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ABSTRACT BOOK

Coordinated by:
Prof. Dr. Ioan Sporea
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President 18th ESBRA Meeting
Ioan Sporea

President ESBRA
Sebastian Mueller

Invited talks

1. Alcoholic hepatitis – from diagnosis to treatment	Phillipe Mathurin
2. Alcohol and microbiome in ALD	Gyongyi Szabo
3. Transcranial Magnetic Stimulation in addiction therapies	Marco Diana
4. Novel medications and addiction	Lorenzo Leggio
5. Alcoholic cirrhosis and reversibility	Massimo Pinzani
6. Losing and regaining control over alcohol intake	Rainer Spanagel

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Prof. Dr. Alis Dema

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Transcriptional Changes in the Pathological Divergence of Acute Alcoholic Hepatitis and Cirrhosis

Brandon J. Peiffer and Zhaoli Sun (Johns Hopkins School of Medicine, Department of Surgery, Baltimore, MD USA)

Alcoholic hepatitis (AH) is a life-threatening liver disease characterized by leukocyte infiltration and hepatocyte necrosis. Frontline treatments for AH include steroids and nutritional support. However, patients with chronic/refractory AH that does not respond to steroids may progress to end-stage AH, where liver transplantation is the only life-saving measure. To better understand the unique pathology of patients with alcoholic hepatitis, whole-liver tissue samples from our AH repository were selected for transcriptomic and proteomic analysis. Samples from donor liver tissue and a selection of six different end-stage liver diseases were used as control. These ESLDs include alcoholic cirrhosis (AC), autoimmune hepatitis (AIH), hepatitis B virus (HBV), hepatitis C virus (HCV), primary biliary cholangitis (PBC) and Non-alcoholic Steatohepatitis (NASH).

Differential expression analysis and principal component mapping of RNAseq data show that gene signatures from all disease-states can be separated from non-diseased (donor) samples, indicating several commonalities in molecular programs of liver injury. Moreover, we could identify several unique differentially-expressed genes for all seven ESLDs. These unique transcriptomic signatures can be used to guide future therapy and diagnostics. Proteomic mass spectrometry and phenotypic analysis of the RNAseq data provided insight into the cellular composition of these disease states. Where results show disease-specific changes in immune cell infiltration, fibrogenesis, and regeneration. Alcoholic liver disease (AH and AC) patients exhibit a predominance of fibrogenic cells. However, these two diseases are distinguishable by key differences in leukocyte infiltration. Sample data from both AH and AC were well defined, and showed key changes in both RNA and protein expression. These changes may allow us to better understand a patient's likelihood of pathological progression in alcoholic liver disease (ALD).

YAP AND LIVER REGENERATION IN PATIENTS WITH SEVERE ALCOHOLIC HEPATITIS

Line Carolle Ntandja-Wandji[1], Mohamed Bou Saleh[1], Alexandre Louvet[1], Emmanuel Boleslawski[2], Pau Sancho-Bru[3], Josepmaria Argemi[4], Sébastien Dharancy[1], Ramon Bataller[4], Philippe Mathurin[1], Laurent Dubuquoy[1] (1 - Univ. Lille, Inserm, CHU Lille, U1286 - INFINITE - Institute for Translational Research in Inflammation, F-59000 Lille, France; 2 - Univ. Lille, Inserm, CHU Lille, U1189 - ONCO-THAI - Image Assisted Laser Therapy for Oncology, F-59000 Lille, France; 3 - Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), University of Barcelona, Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Barcelona, Spain; 4 - Division of Gastroenterology, Hepatology and Nutrition. Pittsburgh Liver Research Center. University of Pittsburgh Medical Center (UPMC), Pittsburgh, PA, USA)

Introduction:

Alcoholic hepatitis (AH) is a complex life-threatening disease associated with a profound defect of liver regeneration. To decipher cellular mechanisms involved in this defect we focused on the Hippo/YAP pathway which has been shown to play a role in liver regeneration.

Patients and methods:

The Hippo/YAP pathway was dissected in explants of patients transplanted for AH or

alcohol-related cirrhosis and in control livers, using RNA-seq, real-time PCR, western blot, immunohistochemistry and transcriptome analysis after laser microdissection. We also transfected primary human hepatocytes with constitutively active YAP (YAPS127A) and treated primary hepatocytes isolated from AH livers with a YAP inhibitor. Finally, we used mouse models of ethanol exposure (Lieber de Carli) and liver regeneration (carbon tetrachloride) after hepatocyte transduction of YAPS127A.

Results:

In AH samples, RNA-seq analysis and immunohistochemistry of total liver and microdissected hepatocytes revealed marked downregulation of the Hippo pathway and abnormal activation of YAP in hepatocytes. Overactivation of YAP in hepatocytes in vitro and in vivo led to biliary differentiation and loss of key biological functions such as regeneration capacity. Conversely, a YAP inhibitor restored the mature hepatocyte phenotype in abnormal hepatocytes taken from patients with AH. In ethanol-fed mice, YAP activation using YAPS127A resulted in a loss of hepatocyte differentiation. Hepatocyte proliferation was hampered by YAPS127A after carbon tetrachloride intoxication.

Conclusion:

Aberrant activation of YAP plays an important role in hepatocyte transdifferentiation in AH, through a loss of hepatocyte identity and impaired regeneration. Thus, targeting YAP is a promising strategy for the treatment of patients with AH.

Keywords: Alcoholic Hepatitis – Liver – Regeneration - Hippo/YAP – Hepatocytes - Transdifferentation

Toll-like receptor 4 inhibition acts synergistically with G-CSF to prevent organ injury and induce liver regeneration in acute-on-chronic liver failure

Cornelius Engelmann, Fausto Andreola, Abeba Habtesion, Simone Novelli, Annarein JC Kerbert, Nathan Davies, Sofia Ferreira-Gonzalez, Stuart Forbes, Thomas Berg, Rajiv Jalan

Background and Aims:

Acute-on-chronic liver failure (ACLF) is characterised by lack of regeneration. Granulocyte colony stimulating factor (G-CSF) carries pro-regenerative properties and has been shown to be of benefit in ACLF. However, the large trial of G-CSF (GRAFT study) in patients with ACLF showed no benefit and in certain groups mortality tended to be higher. This study was performed to define the mechanisms underlying the negative effect of G-CSF and determine whether its beneficial effect could be harnessed using a toll-like receptor 4 (TLR4) antagonist.

Method:

Two mouse models of ACLF were used: CCL4 (0.5mg/ml,6w) to induce chronic liver injury followed by LPS i.p. (Klebsiella,4mg/kg) (n=4-10) or Galactosamine (GalN) i.p. (1000mg/kg) as a second hit (n=8). 1h after, G-CSF 250µg/kg s.c. and/or TLR4-inhibitor TAK-242 10mg/kg i.p. were injected and continued every 24h. The treatment duration was 24h and 5d in the LPS model and 48h in the GalN model. Samples were stored and analysed for liver injury, inflammation, senescence and regeneration.

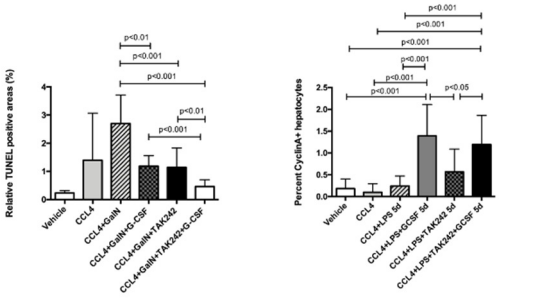
Results:

6w CCL4 led to bridging fibrosis, TLR4 up-regulation and infiltration of G-CSFr expressing cells. LPS increased ALT levels, cell death (TUNEL+), enhanced hepatic infiltration of neutrophils (Ly6G+), macrophages (F4/80+) and TNFa. G-CSF increased the 48h mortality from 0% to 66%, aggravated liver inflammation with macrophage and NK cell (CD45+, CD49b+, CD3-, CD19-) infiltration and IL6 expression. G-CSF+TAK-242 reduced the mortality to 0%, abrogated the liver injury (TUNEL) and liver inflammation (macrophages, neutrophils, TNFa, IL6) significantly. In the second model, GalN also induced a significant liver injury. Treatment with G-CSF+TAK-242 was significantly more effective than the individual therapies (figure). G-CSF+TAK-242 was associated with increased liver regeneration evidenced by increased tissue expression of pSTAT3 and BCL2. CCL4+LPS induced a p53 and p16-dependent cell cycle arrest and lack of proliferation (CyclinA) in hepatocytes. G-CSF+TAK-242 mitigated senescence and significantly increased the rate of CyclinA expressing hepatocytes (figure) suggesting enhanced liver regeneration.

Conclusion:
The present study shows that G-CSF is deleterious in LPS-associated ACLF through further activation of inflammatory pathways and immune cell infiltration. TLR4 inhibition with TAK-242 prevented G-CSF driven tissue injury and induced liver regeneration showing evidence of synergy between the two molecules thereby providing a novel therapeutic strategy for ACLF patients.

Figure:

Figure: Anti-apoptotic (TUNEL) and pro-proliferative (CyclinA) effect of G-CSF plus/minus TAK-242 in preclinical models of acute-on-chronic liver failure



S 2 New mechanisms involved in alcohol use disorder and new therapeutics: insights from the preclinical side

The symposium will present new hypotheses regarding neurobiological and biological mechanisms involved in alcohol use disorder and new potential therapeutical interventions such as brain stimulation and psychedelics in animal models.

Chairs
Prof M Naassila. Research Group on Alcohol & Pharmacodependences GRAP, INSERM UMRS_1247, Amiens, France.
Dr Sebastien Carnicella. Grenoble Institut des Neurosciences (GIN), France.

Speakers
Dr Sami Ben Hamida. University of Strasbourg. ***Role of GPR88 in alcohol addiction and as a new therapeutic target.***
Pr Vincent Van Waes. Laboratoire de Neurosciences Intégratives et Cliniques - EA481, Université Bourgogne Franche-Comté, Besançon – France. ***Transcranial direct current stimulations (tDCS) reduce motivation to drink ethanol and reacquisition of ethanol self-administration in mice***
Dr Jérôme Jeanblanc. Research Group on Alcohol & Pharmacodependences GRAP, INSERM UMRS_1247, Amiens, France. ***Behavioral and brain Effects of psilocybin in a relevant animal model of binge drinking.***
M. Raphaël GOUTAUDIER (PhD student). Grenoble Institut des Neurosciences (GIN), France. ***Role of nigrostriatal pathway in motivated behaviors and alcohol use disorder.***

Transcranial direct current stimulation (tDCS) reduces motivation to drink ethanol and reacquisition of ethanol self-administration in mice

Vincent Van Waes (Laboratoire de Recherches Intégratives en Neurosciences et Psychologie Cognitive, Université Bourgogne Franche-Comté, Besançon, France)

Transcranial direct current stimulation (tDCS) is an emerging noninvasive brain neuromodulation technique aimed at relieving symptoms associated with psychiatric disorders, including addiction. The goal of the present study was to better identify in mice which component of alcohol-related behavior (hedonic effect, behavioral sensitization, self-administration, motivation to obtain the drug) might be modulated by repeated anodal tDCS over the frontal cortex (0.2 mA, 20 minutes, twice a day for 5 consecutive days, as used in clinical trials). Our data showed that tDCS did not modulate the hedonic effects of ethanol assessed in a conditioned place preference test (CPP), or the expression of ethanol-induced behavioral sensitization. Interestingly, tDCS robustly reduced reacquisition of ethanol consumption (50% decrease) following extinction of self-administration (operant paradigm). Furthermore, tDCS significantly decreased motivation to drink ethanol on a progressive ratio schedule (30% decrease). Taken together, our results show for the first time a dissociation regarding the effects of tDCS on "liking" (hedonic aspect; no effect in the CPP) and "wanting" (motivation; decreased consumption on a progressive ratio schedule). Our tDCS procedure in animals will allow us to better understand the mechanisms of action of

tDCS and therefore accelerate its development as a complementary and innovative tool to help alcohol-dependent patients maintain abstinence or reduce ethanol intake.

Keywords: Noninvasive brain stimulation, locomotor sensitization, conditioned place preference, oral ethanol self-administration, motivation, operant paradigm

Reduction of alcohol consumption by psilocybin: role of serotonin type 2A receptors in the nucleus accumbens and identification of gene regulations by PCR array

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After falling out of favor in the 1960s, psychedelics are making a comeback in the treatment of psychiatric and addictive disorders. A proof of concept was published in 2015 showing that psilocybin sustainably (36 weeks) reduces alcohol consumption in patients with alcohol use disorder (AUD). Our latest unpublished results show that acute administration of psilocybin reduces not only relapse after abstinence but also alcohol consumption before withdrawal. Interestingly, we show that this effect of psilocybin is mediated by type 2A receptors in the nucleus accumbens in an experiment where psilocybin and/or ketanserin (5-HT2A antagonist) are injected intraperitoneally, or in the nucleus accumbens or in the ventral tegmental area. Using a PCR array technique, we screened 100 genes having a role in the neurotransmission of the nucleus accumbens, genes potentially involved in the effects of psilocybin; in particular genes of glutamatergic, serotonergic, dopaminergic and gabaergic neurotransmissions. The current experiments aim at analyzing the epigenetic modifications (methylation, acetylation) that can explain the gene regulations induced by psilocybin. Our project brings major results in the understanding of the neurobiological mechanisms underlying the beneficial effects of psilocybin in the treatment of AUD and reinforces the interest to conduct new clinical trials.

Fundings: Era-Net Neuron Psi-Alc - 18-NEUR-0007-01. https://www.psialc.org. G. Fouquet has a postdoctoral research fellowship from the Era-Net Neuron Project funded by the National Research Agency (ANR) in France.

INVESTIGATION OF THE FUNCTION OF NIGROSTRIATAL DOPAMINERGIC NEURONS IN MOTIVATED BEHAVIOR AND ALCOHOL ADDICTION WITH DREADDs

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Key words: Addiction, Alcohol, Motivation, Dopamine, Nigrostriatal pathway, DREADDs, Electrophysiology, Microdialysis, Behavior.

Alcohol addiction is a chronic relapsing disorder characterized by compulsive seeking and drinking of high amount of alcohol, despite knowledge of negatives associated consequences[1]. A critical point explaining relapse is the apparition of a negative emotional state during withdrawal including notably depression, anxiety and apathy. Dopamine (DA), a key modulator of motivational and reward processes, has long been thought to be involved in addiction, but despite extensive research, its exact contribution to alcohol addiction remains unclear. Previous results from our team, and others, suggest a crucial role of the nigrostriatal DA pathway in the control of affective and motivated behaviors[2,3,4]. In this project, we therefore investigated DA level of the nigrostriatal DA pathway of animals expressing, or not, a compulsive alcohol-related behavior. Then, we ask whether the alteration of this pathway leads to a negative emotional and motivational state reminiscent of alcohol withdrawal and whether this alteration exacerbates compulsive alcohol-related behavior.

To induce a selective and reversible alteration of the nigrostriatal DA pathway, we used the chemogenetic approach called Designer Receptors Exclusively Activated by Designer Drugs (DREADDs)[5]. In transgenic TH-Cre rats, we selectively transduced SNc DA neurons with the inhibitory designer receptor hM4Di. To activate this receptor, we choose the synthetic designer drug C21 and we performed in vivo electrophysiology and microdialysis experiments to validate that our approach induced a reliable and selective hypodopaminergia of the nigrostriatal pathway[6]. Then, we assessed the effect of this experimental hypodopaminergia on motivation, as well as on anxiety- and depression-related behaviors. Finally, we trained rats to self-administer alcohol, we identify rats that do not express compulsive alcohol-related behavior and we tested whether the induction of a hypodopaminergic state, in these animals, led to the development of the compulsive alcohol-related behavior.

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S 3 Measuring alcohol consumption

The role of biomarkers in the measurement of alcohol intake – what is new and what do the German evidence and consensus-based guidelines recommend in terms of application

Friedrich M. Wurst*, Erika Baum, Gallus Bischof, Eva Hoch, Karl Mann, Tim Neumann, Oliver Pogarell, Hans Jürgen Rumpf, Ulrich W. Preuss, Claudia Spies, Natasha Thon, Wolfgang Weinmann, Marc Luginbühl, Falk Kiefer, Anil Batra, Sabine Hoffmann (*Speaker)

Background

Screening and measurement of alcohol intake are of utmost importance for early identification and intervention in alcohol use disorders and other contexts where alcohol use is not appropriate.

Besides questionnaires such as the AUDIT, in the last decades, direct ethanol metabolites have proven high evidence and therefore are recommended also by guidelines. The practical application of the tests has in the last years reached a routine level, so that the markers can be used in everyday routine almost everywhere.

Method

After an introduction to the topic, results and recommendations of the German S3 Guideline Alcohol (2020) which is based on the 2014 guideline will be presented. In both cases, a systematic review was performed. In 2014 one hundred and seven of 1869 publications have been considered relevant and in 2019 (restricted to meta-analyses and systematic reviews) of 11 publications in the last 5 years, 2 MA and 6SRs have been assessed as relevant.

Results

The high levels of recommendation for the direct ethanol metabolites (mostly A) have been confirmed in the current guideline. Especially for phosphatidylethanol (PEth) the database has increased significantly in the last years. Also, a new recommendation regarding the use of PEth for screening during pregnancy (FASD) has been added.

Discussion and Conclusion

A rich body of evidence strengthens the positions of direct ethanol metabolites in the measurement of alcohol use. Besides the clear recommendations, now for a widespread use of the guidelines the implementation through various organisations is crucial. Furthermore questions in the context of clinical use, establishing cut-offs and issues regarding analytical caveats (purity of reference substances etc.) will be discussed.

Measuring alcohol consumption

Roberta Agabio (Department of Biomedical Sciences Section of Neuroscience and Clinical Pharmacology University of Cagliari, Italy)

Still today, training in alcohol use and misuse is lacking and many health professionals are unable to quantify alcohol consumption using the alcoholic unit. Specialists in the field of alcohol use and misuse, on the other hand, use this unit of measurement to assess whether alcohol consumption exceeds the quantities defined as at low risk based on the individual characteristics of patients, such as sex, age, physiological situations (pregnancy and breast-feeding) and/or pathological conditions (liver disease, mental disorders, etc.) and drinking pattern (number of alcoholic units consumed on a single occasion, daily, weekly, fasting, during meals). In addition, specialists in the field use the alcoholic unit to measure any change in alcohol consumption following the implementation of interventions aimed at reducing consumption or achieving abstinence from alcohol.
Additionally, because of the lack of awareness on how to quantify alcohol consumption, many health professionals record the information collected during the medical history on alcohol consumption using hard to interpret generic expressions, such as moderate or excessive drinkers. If this terminology is not accompanied by the number of alcoholic units consumed over a specific time interval (single occasion, within a few hours, a day, a week), they do not allow for any type of evaluation.
This lesson will aim to provide participants with the relevant knowledge and skills to allow them to quantify alcohol consumption into alcoholic units. In addition to the alcoholic unit, the lesson will provide the threshold values of alcohol consumption - expressed in alcoholic units - to divide the population into different categories, based on alcohol consumption, i.e., abstainers, at low-risk and high-risk drinkers (for single occasion, daily, weekly).
Once these skills have been acquired, the lesson will focus on the characteristics of the concentration of alcohol in the blood (blood alcohol levels), including the factors that influence the latter, its changes over time and the behavioral effects related to its different values.
These skills are essential for the identification of excessive consumption and represent the basis for acquisition of the subsequent information provided by the course "Alcohol use and misuse: from neurobiology to clinical practice".

Diagnosis of alcohol use disorder and identification of unhealthy alcohol consumption

Anne Lingford-Hughes

Animals models used to study alcohol use disorder

Rainer Spanagel

S4 COVID and Alcohol Use Disorder

COVID-19 AND ALCOHOL: GAINS AND LOSSES

Jonathan Chick (Castle Craig Hospital, Scotland, UK)

At the outset of the pandemic, some harms resulted from the myth that drinking alcohol might prevent illness. However, heavy alcohol consumption can worsen acute pulmonary infections, although a large population study in the UK did not find worse COVID illness outcomes in heavy drinkers (while obesity, smoking and lack of exercise were aggravations). It is possible, but not definite, that heavy drinking may reduce immune response to vaccination. Some countries banned or restricted sales of alcohol and South African research showed consequent reduction in violent deaths and serious injuries, although for periods curfew was also a factor in that result. Lockdown was emotionally stressful for some, and some appear to have increased their drinking (and drug use) with possible increases in 'deaths of despair'. Other people reduced drinking perhaps due to fewer social occasions and pub closures. In many countries, total sales altered little, as supermarket sales matched the reduction in pub and restaurant sales. It was possible with efficient quarantining for some treatment centres to remain active, but many closed. Effects of the pandemic on mortality in drinkers will be discussed by other speakers.

Impact of COVID-19 confinement on alcohol purchases in Great Britain: controlled interrupted time-series analysis during the first half of 2020 compared with 2015-2018

Peter Anderson

The effect of Covid-19 on alcohol use disorder and the role of universal alcohol screening in an inpatient setting: a retrospective cohort control study

Mohsan, Subhani[1,2], Abhishek Sheth[1,2], Stuart Unitt [4], Guruprasad P. Aithal [1,2], Stephen D Ryder[1,2], Joanne R Morling[1,2,3] ([1]Nottingham Digestive Diseases Biomedical Research Centre (NDDC), School of Medicine, University of Nottingham; [2]NIHR Nottingham Biomedical Research Centre, Nottingham University Hospitals NHS Trust and the University of Nottingham, Nottingham, UK; [3]Division of Epidemiology and Public Health, Nottingham University Hospitals NHS Trust and the University of Nottingham, Nottingham, UK; [4]Activity & Access team, Nottingham University Hospitals NHS Trust)

Aim

To assess the impact of Covid-19 on alcohol use disorders (AUD) and the role of universal alcohol screening (UAS) in an inpatient setting.

Methods

Retrospective cohorts were defined as pre-pandemic and pandemic admitted to Nottingham University Hospitals (April to October; 2019 and 2020) and had alcohol assessment by AUDIT-C. AUDIT-C score was assessed against age, sex, ethnicity, admission type, speciality, and primary diagnosis of mental disorders. Subgroup analysis for Covid-19 positive patients was performed.

Results

63,927 admissions (47,954 patients) were included. The pandemic period compared to pre-pandemic had fewer overall admissions (27,349 vs 36,578, p<001), fewer with AUD (17.6% vs 18.4%, p=0.008) but a higher proportion of alcohol dependents (3.7% vs 3.0%, p<0.0001). In the pandemic those with AUD were more likely to be male (p=0.003), white (p<0.001), in relationship (p<0.001), of higher socioeconomic background (p<0.001), have alcohol related mental disorders (p=0.002), emergency admission (p<0.001), medical speciality admission (p<0.001) and shorter length of stay (p<0.033) compared to pre-pandemic AUD. Covid-19 positive patients with concomitant AUD died at younger age (p<0.05) than Covid-19 positive patients at low risk for AUD.

Conclusions

The pandemic changed the characteristics of inpatients with AUD. There was a higher proportion of alcohol dependent admissions with evidence that a younger, less deprived group have been significantly impacted. UAS provides a useful tool to screen for AUD and to identify the change when facing sudden health crises.

Lockdown drinking: how alcohol consumption trends have responded to the pandemic

Dr Sadie Boniface (Head of research, Institute of Alcohol Studies, Visiting researcher, Institute of Psychiatry, Psychology and Neuroscience, King's College London, UK)

Changes in alcohol consumption were one of the indirect effects following the introduction of non-pharmaceutical interventions to reduce the spread of COVID-19 (such as the closure of services and non-essential businesses, restrictions on movement, aka 'lockdowns'). Multiple surveys found the proportion of people in the UK drinking at increasing risk levels rose. This issue attracted considerable media attention during the first lockdown. Broadly similar patterns have persisted since, across several studies. These have translated into alcohol harm, including a 20% increase in alcohol specific deaths and a 14% increase in unplanned admissions for alcoholic liver disease. There is uncertainty around future alcohol consumption trends, with implications for health, which we are exploring in an ongoing collaboration with HealthLumen and Public Health England.

Treatment and prevention strategies during lockdown

Peter Rice

S 5 New insights into neuroinflammatory processes induced by ethanol exposure during early or late brain developmental periods

CANNABINOIDS MODULATE COGNITIVE DEFICITS AND NEUROINFLAMMATION INDUCED BY EARLY ALCOHOL EXPOSURE.

Valverde Olga[1], Alba García-Baos[1] ([1] University Pompeu Fabra, Department of Experimental and Health Sciences, Barcelona, Spain)
Correspondent author: ferran.sanz@upf.edu

Objectives

Foetal alcohol spectrum disorder (FASD) is the term used to describe the physical, mental and behavioural disabilities induced by prenatal and lactation alcohol exposure (PLAE). Numerous molecular mechanisms might be underlying the alcohol-induced teratogenicity, including neuroinflammatory reactions and the alterations of the endocannabinoid system. In this regards, cannabidiol modulates endocannabinoid system and also appears to produce anti-inflammatory effects. In addition, the role of endocannabinoids in the modulation of neuroinflammation has been well documented, acting on different targets, including the PPAR system. In this context, our study aims i) to assess whether cannabidiol could ameliorate cognitive impairments in PLAE mice and ii) to study whether the interaction between endocannabinoids and PPAR system might induce anti-inflammatory effects that would regulate cognitive impairment in FASD-like mouse model.

Materials and methods

For that, to achieve the first objective, we used a model of alcohol binge drinking during gestational and lactation periods in pregnant C57BL/6 female mice (1). Following the prenatal and lactation alcohol exposure, we treated the male and female offspring with cannabidiol from post-natal day (PD) 25 until PD34, and we evaluated their cognitive performance at PD60 (2). For the second objective, we used the same procedure, and we treated offspring with the FAAH inhibitor URB597 and the PPARγ antagonist GW9662 during the same period (PD25 to PD34), and then at PD60, we evaluated cognitive performance and pro-inflammatory markers.

Results

Our results showed that cannabidiol and URB597 treatment during peri-adolescence period ameliorates cognitive deficits observed in our FASD-like mouse model, without sex differences. Moreover, the beneficial effects of URB597 were prevented by the treatment with the PPAR□ antagonist. Additionally, cannabidiol restored levels of TNFα and IL-6 in the hippocampus.

Conclusions

In conclusion, our study provides new findings about the participation of endocannabinoid system in behavioural and molecular pathological mechanism underlying FASD. Our data also suggests that cannabidiol could represent a therapeutic agent to counteract cognitive impairments and neuroinflammation in FASD.

Keywords

FASD, cannabidiol, endocannabinoids, inflammation, PPARγ

References

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ROLE OF EXOSOMES AS BIOMARKERS OF THE NEUROINFLAMMATION INDUCED BY ADOLESCENT BINGE DRINKING

María Pascual[1], Francesc Ibáñez[2], Consuelo Guerri[2] ([1] Department of Physiology, School of Medicine and Dentistry, University of Valencia, Valencia, Spain; [2] Principe Felipe Research Center, Valencia, Spain)

Current studies evidence the role of miRNAs in extracellular vesicles (EVs) as key regulators of different biological and pathological events, including neuroinflammatory and neurodegenerative processes. We have demonstrated that ethanol can induce an inflammatory immune response through the toll-like receptor 4 (TLR4) activation, and that the female brain is more vulnerable to the inflammatory effects of binge ethanol drinking in adolescence than the male brain. Considering the capacity of EVs to cross the blood-brain barrier and their high

stability in peripheral circulation, we evaluated the potential gender differences in the levels of inflammatory-related miRNAs in plasma EVs derived from alcohol-intoxicated adolescents. MiRNAs (e.g., mir-146a-5p, mir-21-5p, mir-182-5p, mir-183-5p) of human and murine plasma EVs derived from female and male adolescents intoxicated with or without ethanol were analyzed by RT-PCR. Short- and long-term effects of ethanol intoxication in adolescence were also evaluated in the expression of some miRNAs and their related genes in cerebral cortex. We demonstrated that while alcohol intoxication lowers anti-inflammatory miRNA (mir-146a-5p, mir-21-5p, mir-182-5p) levels in plasma EVs from human and mice female adolescents, these EV miRNAs increased in males. In mice brain cortices, ethanol treatment lowers mir-146a-5p and mir-21-5p levels, while triggering a higher expression of inflammatory target genes (Traf6, Stat3, and Camk2a) in adolescent female mice. These results indicate that female and male adolescents differ in their plasma EV miRNA profile upon ethanol intoxication, suggesting that female adolescents are more vulnerable than males to the ethanol-induced inflammation. These findings also support the role of circulating miRNAs in EVs as biomarkers to screen neuroinflammation and brain damage.

Keywords: Exosomes/extracellular vesicles, neuroinflammation, adolescence, alcohol, binge drinking, microRNAs.

BINGE-DRINKING, INFLAMMATION AND GLUN2B SUBUNITS: A MENAGE A TROIS TO EXPLAIN COGNITIVE DEFICITS AND HIPPOCAMPUS PLASTICITY DYFUNCTIONS DURING ADOLESCENCE IN RATS

Oliver Pierrefiche, Chloé Deschamps, Catherine Vilpoux and Mickael Naassila (INSERM UMR1247, Groupe de Recherche sur l'Alcool et les Pharmacodépendances, Université de Picardie Jules Verne, Amiens - France)

Ethanol-induced neuroinflammation has a major role in the effects of alcohol in the brain, notably during adolescence when the brain is not fully mature. Indeed, the so-called binge drinking behaviour, very popular in the young population, is known to induce both short and long-lasting cognitive deficits. However, the effects of only few binges on learning and memory are underestimated while the mechanism of action of ethanol during the very first exposure to ethanol during adolescence is not enough studied. In our recent work, our goal was to investigate whether ethanol-related neuroinflammatory processes were part of the delayed cognitive deficits and concomitant alterations of synaptic plasticity in the hippocampus of adolescent rats after only two binge-like episodes. Male adolescent rats were administered two ethanol ip injection (3 g/kg, v/v) at 9h interval, with or without anti-inflammatory agents (minocycline or indomethacin) and all experiments were conducted 48h later. Synaptic plasticity was measured on hippocampus slices and a GluN2B antagonist was tested. Learning was tested with novel object recognition task. Microglial reactivity, Toll-like receptor 4 (TLR4) expression, a receptor of the neuroimmune system, and mRNA levels for several cytokines were assessed at 48h. The results indicate that two binge-like ethanol exposure abolished long-term synaptic depression in the hippocampus and blocked learning after 48h. The anti-inflammatory agents prevented both behavioral and synaptic effects of ethanol and a blocker of the GluN2B subunit restored synaptic plasticity. At 48h, there was no microglial reactivity and TLR4 expression was reduced in neurons. These results suggest that ethanol-induced neuroinflammation occurs transiently during the binges and that modulating neuroinflammation but also glutamatergic transmission could be a promising way to prevent or reverse the effects of few binge drinking on cognitive processes in rats.

PET imaging for preclinical investigation of ethanol-induced neuroinflammation in adolescent animals

Wadad Saba (Université Paris-Saclay, CEA, CNRS, Inserm, Biomaps, Service Hospitalier Frédéric Joliot, 4 place du général Leclerc, 91401 ORSAY France)

A large body of preclinical research has shown that neuroimmunity plays a key role in the deleterious effects of alcohol on the brain and highlighted the vulnerability of adolescent

brain to alcohol neurotoxicity. Translational imaging techniques are needed to monitor the efficacy of strategies to prevent or mitigate neuroinflammation and alleviate alcohol-induced neurotoxicity in vivo. We recently demonstrated, using Positron Emission Tomography (PET) imaging with the translocator protein 18 kDa (TSPO) radioligand [18F]DPA-714, an immediate and prolonged glial activation among brain regions after an acute and initial ethanol exposure in baboon. An increase in the brain uptake of [18F]DPA-714 was also observed after repeated ethanol exposure in adolescent rats confirming neuroimmune response. Our results showed that pre-treatment with the opioid and toll-like receptor antagonist nalmefene significantly alleviated the neuroimmune response to ethanol exposure in all brain regions. More recently, we investigate the role of nicotinic receptors in the ethanol related neuroinflammatory response and suggest a possible implication.

S 6 EASL ESBRA joint symposium: Epidemiology of ALD: What are the real numbers?

Policies to reduce the burden of ALD

Nick Sheran

Epidemiology of alcohol-related liver disease in Europe

Peter Jepsen

Non-invasive biomarker screening and alcohol-related liver disease in the general population

Maja Thiele, MD, PhD, associate professor at Fibrosis, fatty liver and steatohepatitis research centre Odense (FLASH), Odense University Hospital and University of Southern Denmark.

Screening for liver fibrosis has the potential to change the current approach from diagnosing alcohol-related liver diseases late, when patients have already developed complications of cirrhosis, to diagnosing liver fibrosis in asymptomatic subjects. Early disease detection could provide an excellent opportunity for preventing disease progression by enabling improved alcohol rehabilitation and treatment of comorbid metabolic risk factors. This talk will examine possibilities and barriers of screening for liver fibrosis in a population with harmful use of alcohol, including burden of disease, population prevalence of 'hidden' significant liver fibrosis, serum and imaging modalities, as well as positive and negative consequences of screening.

Non-invasive fibrosis assessment

Manolis Tsochatzis

S 8 Translational Neuroimaging in Alcoholism I: Novel approaches to neuro-immune and functional networks

Alcohol, Microbiota and Brain function

Sophie Leclercq

Advanced diffusion-weighted MRI to identify glia responses in the alcoholic brain

Silvia De Santis[1,2] ([1]Instituto de Neurociencias, CSIC/UMH, San Juan de Alicante, Alicante, Spain; [2]CUBRIC, School of Psychology, Cardiff University, Cardiff, UK)

Introduction: Neuroinflammation is increasingly implicated in the pathophysiology of several psychiatric disorders and, importantly, has been proposed as a mechanism of alcohol-related brain damage [1]. We recently used diffusion tensor imaging (DTI) to demonstrate widespread diffusivity alterations in the brain of chronically drinking humans and rats, associated with a microglial reaction [2]. However, DTI parameters, while sensitive to overall diffusion changes, lack specificity to dissect the contribution of specific cellular compartments like glia or neurons [3]. Here, we present a non-invasive diffusion-weighted MRI (dw-MRI) approach to image glia activation *in*

vivo. We used well-characterized rat models of inflammation (lipopolysaccharide) and inflammation with degeneration (ibotenic acid) to prove sensitivity and specificity of the imaging biomarkers to microglia and astrocytes. We demonstrate feasibility of the method in a cohort of healthy humans.

Materials and Methods: 36 rats were injected bilaterally in the dorsal hippocampus (left:toxin; right:saline). Different post-surgery waiting time, and a cohort of animals treated with the microglia depleter PLX5622, were used to isolate the following conditions: a) microglia activation, b) astrocyte activation, and c) microglia activation with neuronal death. The animals were then scanned in a Bruker 7T MRI using a dw-MRI sequence to extract imaging biomarkers (microglia ramification proliferation, astrocyte body volume and tissue fraction), and immediately perfused for ex-vivo immunohistological analysis of microglia (Iba-1+), astrocytes (GFAP+), and neurons (NeuN+). The same dw-MRI protocol was applied in 6 healthy volunteers.

Results: We found a strong association between imaging and immunohistochemistry biomarkers, and specifically: a significant retraction of microglia ramifications in condition a), increase in astrocyte volume in condition b), and retraction of microglia ramifications with decrease of tissue fraction in condition c). We prove the translational value of the approach reporting significant correlations between imaging and histological microglia markers measured across different brain regions in humans [4].

Conclusions: The proposed imaging framework can detect neuroinflammation in vivo and non-invasively, dissociate a microglial reaction from an astrogliosis, and reveal the presence of a concomitant neuronal loss. As such, it holds the potential to transform alcohol research by providing a tool to clarify the role of inflammation in both preclinical models and clinical investigations.

Keywords: neuroinflammation, MRI biomarkers, gliosis, alcohol-induced brain damage

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ADVANCED NETWORK ANALYSIS OF CFOS RESPONSES REVEALS DISTINCT BRAIN STATES FOR ALCOHOL AND SWEET MEMORY RECALL

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Objectives

Cue-reward associations form distinct memories that can drive appetitive behaviors and cravings for both drugs and natural rewards. It is still unclear how such memories are encoded in the brain's reward system.

Materials and methods

We trained rats to concurrently self-administer either alcohol or a sweet saccharin solution as drug or natural rewards, respectively. Memory recall due to cue exposure reactivated reward-associated functional ensembles in reward-related brain regions, marked by a neural cFos response. As shown in a previous study the local ensembles activated by cue presentation for either reward consisted of similar numbers of neurons [1]. Here we used advanced statistical network analysis and we found robust

reward-specific co-activation patterns across brain regions.

Results

Interestingly, the resulting meta-ensemble networks differed in several things: 1) the connectivity strength and edge-weight distributions, 2) the modular structure of the functional network, which seems to be strongly affected by alcohol; 3) communication efficiency in the network is significantly smaller in case of alcohol; 4) and the most influential regions, which in case of saccharin comprised the prefrontal cortex, while for alcohol seeking control shifted to insular cortex with strong involvement of the amygdala.

Conclusions

Our results support the view of memory representation as a differential co-activation of local neuronal ensembles [2].

Keywords

Network analysis Reward seeking Prefrontal cortex Insular cortex Neuronal ensembles

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High-density neurometabolic mapping and network analysis in alcohol dependent rats

Cornelius C. W. Willacey

S 9 Acute-on-chronic Liver Failure (ACLF) in Alcoholic Hepatitis Patients

Acute on chronic liver failure in alcholic hepatitis

Christophe Moreno, MD, PhD (CUB Hôpital Erasme, Université Libre de Bruxelles, Brussels, Belgium)

Alcoholic hepatitis is a clinical syndrome characterised by the recent onset of jaundice in a context of chronic heavy alcohol use. Other signs of liver decompensation (ascites and/or encephalopathy) can be present. Histologically, steatosis, hepatocyte ballooning and inflammatory infiltrate with polymorphonuclear neutrophils are the typical lesions observed. Blood analysis reveals neutrophilia, increased bilirubine level (>5 mg/dL), AST > 50 IU/L, although rarely above 300 IU/L, and an AST/ALT ratio greater than 1.5-2. In severe forms of AH, prolonged prothrombin time, low platelet count and hypoalbuminemia are frequently present (1).

Different prognostic models have been developed, aiming to identify patients at high risk of early death. The Maddrey's discriminant function (mDF) or the Model for End-Stage Liver Disease (MELD) are the scores most often used in clinical practice and in clinical trials to assess disease severity. A mDF ≥ 32 or A MELD > 20 are associated with poor short-term prognosis and are usually the thresholds to initiate specific therapy (2) (3).

General therapy includes providing adequate calorie and protein intake, as well as vitamins (i.e. thiamine, folate, and pyridoxine) and preventing withdrawal syndrome in patients with alcohol dependence. In the absence of contra-indications, corticosteroids are currently indicated in patients with severe forms of AH. The short-term survival benefit of corticosteroid treatment has been confirmed in a recent meta-analysis of individual data. However, the survival benefit associated with corticosteroids is modest and transient as the benefit does not persist beyond 1 month. This recent meta-analysis also confirmed that pentoxifylline is ineffective (4).

In patients treated with corticosteroids, early improvement in liver function has a major impact on short-term mortality. The Lille model, which is based on pretreatment data plus the response of serum levels of bilirubin to a 7-day course of corticosteroid therapy ranges from 0 to 1, with a score ≥ 0.45 indicating non response to corticosteroids. Non-responders to corticosteroids have a very poor prognosis, with a 6-month survival rate between 20-30% (5).

Although challenging the 6-month rule of alcohol abstinence before access to LT, early

LT for severe AH patients non responders to medical therapy, is the only effective therapy in highly selected patients (first liver decompensation, absolute consensus of medical and paramedical team). Different studies from Europe and North America assessing early LT in severe AH patients demonstrated dramatic improvement of survival compared to non transplanted patients. Rate of alcohol relapse appear to be similar in patients undergoing early LT to those patients transplanted after a period of abstinence (6) (7).

Acute-on-chronic liver failure (ACLF) is a recently defined entity that occurs in patients with cirrhosis and is characterised by acute deterioration, organ failures, and a high risk of short-term mortality. Currently different definitions have been created by several scientific societies (the Asian Pacific Association for the Study of the Liver [APASL], the European Association for the Study of the Liver – Chronic Liver Failure consortium [EASL-CLIF], the North American Consortium for the Study of End-Stage Liver Disease [NACSELD].

The EASL-CLIF consortium proposed a definition and diagnostic criteria for ACLF based on a large multicentre European cohort including all patients with decompensated cirrhosis, the CANONIC study (8). According to this study, the grade of ACLF was defined by the number of organ failures defined by the CLIF Consortium Organ failure score (CLIF-C OFs) (an adapted and simplified version of the SOFA score), and the presence of kidney and/or neurological dysfunction.

Why is ACLF relevant in alcoholic hepatitis?

In the CANONIC study, alcohol-related cirrhosis (AC) with active alcohol consumption represented approximately 25% of the cases of ACLF. Interestingly, although liver biopsy results were not reported, laboratory features and the prescription of corticosteroids in a significant proportion of this group of patients highly suggest alcoholic hepatitis (AH) is a frequent cause of ACLF. Moreover, patients with AC and active alcohol consumption more often have ACLF grade 2 and 3 than other patients (8).

More recently, the multicentre, prospective, observational PREDICT study included cirrhotic patients with acute decompensation. Among the precipitating events associated with ACLF development, bacterial infection and severe alcoholic hepatitis were the two most frequently observed events (9).

In a prospective cohort of patients with severe AH, the 28-day cumulative incidence of death in patients without ACLF or with ACLF-1, ACLF-2, or ACLF-3 was 10%, 31%, 58%, and 72%, respectively (10). Whether corticosteroid administration improves short-term survival in patients with severe AH and ACLF is currently unknown as the majority of these patients are excluded from clinical trials. In a Belgian cohort of severe AH patients, the probability of response to corticosteroids using the Lille model was reduced in patients with ACLF and progressively reduced among grades of ACLF (77% for patients without ACLF, 52% for ACLF-1, 42% for ACLF-2, and 8% for ACLF-3) (10). In a subanalysis of the STOPAH trial, a decreased chance of Lille response and greater mortality with higher ACLF grades were also reported. However, the survival benefit of corticosteroids is maintained in Lille responders, irrespective of ACLF grade (11). Therefore, the optimal therapeutic strategy in patients with severe AH and ACLF is still a matter of debate and needs further investigation. Due to the very poor prognosis for these patients, the option of liver transplantation (LT) for patients with ACLF (particularly grade 2 and 3) is frequently considered. Although some groups have recently reported acceptable 1-year survival post-LT in patients with ACLF (including ACLF-3), this topic is highly controversial (12). In addition to the need for strict selection criteria to minimize the risk of alcohol relapse after LT, the question of objective limits beyond which the patient must be considered too sick for LT remains unanswered.

Intensive research evaluating new therapeutic options in ACLF and in severe AH is ongoing, with encouraging preliminary results. Combining efforts between «ACLF specialists» and «AH specialists» is of major importance in order to avoid overlap in clinical trial development.

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Postgraduate Course on ACLF in Alcoholic Hepatitis Patients

Acute on Chronic Liver Failure (ACLF) is a clinically distinct and challenging syndrome resulting from acutely decompensated cirrhosis. ACLF is manifested in severe alcoholic hepatitis (AH) patients and frequently causes multiple organ failures and high mortality. In recent years, the definition and pathophysiologic characterization of ACLF have been refined and translational studies have shed new insights into potential molecular mechanisms of ACLF. This postgraduate course jointly supported by NIAAA and ESBRA, will offer an outstanding platform to learn from four leading experts in ACLF, about current understanding of natural history, treatments and emerging clinical science of the syndrome.

Moderators: Hide Tsukamoto (University of Southern California, USA) and Sebastian Muller (Univ of Heidelberg, Germany)

Opening remarks: George Koob, NIAAA Director or Kathy Jung, Director of Division of Metabolism and Health Effects (NIAAA/NIH, USA) (5 min)

Overview of ACLF

Rajiv Jalan (Royal Free Hospital, UK)

Acutely decompensated cirrhosis refers to the development of ascites, encephalopathy, gastrointestinal hemorrhage, or any combination of these disorders in patients with cirrhosis. ACLF, is a syndrome associated with a high risk of short-term death (i.e., death <28 days after hospital admission) in patients with acutely decompensated cirrhosis. It occurs in the context of intense systemic inflammation, frequently develops in close temporal relationship with proinflammatory precipitating events (e.g., infections or AH), and is associated with single- or multiple-organ failure. Apart from liver transplantation, there are no treatments known to improve survival. Its therapy is therefore an unmet need and currently, eight registered, randomized therapeutic trials are recruiting patients with ACLF.

ACLF in AH

Christophe Moreno (Université Libre de Bruxelles, Belgium)

Acute on chronic liver failure is a frequent and severe complication of alcoholic hepatitis. Prognosis, therapeutic approach and indication for potential early liver transplantation for patients with AH complicated by acute on chronic liver failure, will be discussed during the presentation.

Translational omic analysis of ACLF in AH

Ramon Bataller (Univ Pittsburg, USA)

Recent studies using OMICs techniques such as RNAseq, methylomics, ChIPseq, proteomics and metabolomics in livers and serum/plasma from patients with alcoholic hepatitis has identified molecular mechanisms and biomarkers of liver failure. Hepatocyte-dedifferentiation in these patients occurs by profound fetal reprogramming and loss of function of hepatocyte-enriched transcription factors such as HNF4a. Moreover, these studies revealed profound metabolic reprogramming of the liver with abnormal glucose metabolism. These studies have identified potential novel targets for therapy

Liver transplantation rescues AH patients with ACLF

Alexandre Louvet (Univ Lille, France)

Liver transplantation saves lives in severe AH and explant analysis has enabled better understanding of liver regeneration in this setting. This talk will update the results on liver transplantation for AH & ACLF and discuss the main pathways and drivers associated with a poor outcome. The issue of alcohol relapse will also be discussed.

Exploiting the hypodopaminergic state with Transcranial Magnetic Stimulation in addiction

Diana M. ('G. Minardi' Laboratory of Cognitive Neuroscience, Dept. Chemistry and Pharmacy, University of Sassari, Italy)

Abstract

Repetitive Transcranial Magnetic Stimulation (rTMS) of the dorsolateral prefrontal cortex may affect neuro-adaptations associated with alcohol addiction, potentially influencing drug craving and intake. Previous pre-clinical and clinical evidence suggest a tonically reduced functioning of the mesolimbic dopamine system leading to hypothesize that 'boosting' the hypofunctional system may yield clinical benefits. Here we show that rTMS reduces alcohol and cocaine intake in alcoholics and cocaine addicts. We investigated alcohol intake and dopamine transporter (DAT) availability by Single Photon Emission Computed Tomography (SPECT) in the striatum, in Alcohol Use Disorder (AUD) patients before and after deep rTMS. Fourteen patients underwent baseline clinical and SPECT assessment. Eleven out of 14 patients were randomized into two groups for the REAL or SHAM treatment. Clinical and SPECT evaluations were then carried out after four weeks of rTMS sessions. At baseline, AUD patients showed higher striatal DAT availability than healthy control subjects (HC). Further, patients receiving the REAL stimulation revealed a reduction in DAT availability, whereas SHAM-treated did not. In addition, REAL patients decreased alcohol intake and state anxiety levels. The present results suggest a modulatory effect of deep rTMS on dopaminergic terminals and a potential clinical efficacy in reducing alcohol intake in AUD patients.

Similarly, 18 cocaine addicts (DSM-V) were admitted and randomly assigned to the active or sham stimulation protocol in a double-blind experimental design. They received 12 repetitive TMS r(TMS) sessions 3 times a week for 4 weeks at 100% of motor threshold, over bilateral DLPfc. Cocaine intake (ng/mg) was assessed by hair analysis at baseline (before treatment, T0), after one month (end of treatment, T1) and at 3 (T2) and 6 (T3) months later. All subjects received weekly psycho-logical support. Bilateral TMS of the DLPfC produces a lasting reduction of cocaine-intake significantly more in 10 Hz treated patients vs. SHAM. While further studies are required to confirm these encouraging, preliminary findings they support the notion that DA can be considered a useful biomarker to be targeted by rTMS in addicts.

Key words: Alcohol and cocaine dependence; Dopamine Transporter; Dorsolateral Prefrontal Cortex; Transcranial Magnetic Stimulation; Single Photon Emission Computed Tomography; ^{123}I-FP-CIT; hair analysis.

S 10 The role of inter-organ communication in alcohol induced tissue injury

Alcohol-induced tissue injury in skeletal muscle and adipose tissue; targets or relays for interorgan axis?

Patricia E. Molina, MD, PhD

Description: Chronic hazardous alcohol use impacts metabolic homeostasis producing effects that range from altered fat mass to high incidence of dyslipidemia and metabolic syndrome. Our studies have focused on understanding the impact of chronic binge alcohol (CBA) administration on the metabolic comorbidities associated with HIV. The mechanisms underlying (and preceding) accentuated end-stage wasting in CBA (CBA/SIV) macaques include amplified localized skeletal muscle (SKM) inflammation, profound depletion of SKM antioxidant capacity (oxidative stress), increased SKM proteasomal activity, and decreased myoblast differentiation potential. Our data also show mesenteric adipose tissue structural (decreased adipocyte size and profibrotic milieu), functional (altered lipogenic enzyme expression and inflammation) and secretome (decreased adiponectin release) in CBA/SIV macaques. The impaired SKM and adipose tissue functional phenotype associate with lower insulin sensitivity and reduced insulin release following a glucose load. The ability of SKM and adipose tissue to produce and release myokines, adipokines, and other cellular products including proteins and microRNAs suggests that alcohol-associated muscle and adipose pathophysiology may in turn impact distant organ and tissue homeostasis. Ongoing work in our laboratory examines how alcohol-induced injury in SKM and adipose tissue may act as a relay in interorgan mechanisms underlying pancreatic endocrine dysfunction. Specifically, the potential contribution of miRNAs contained in extracellular vesicles (EVs) is the focus of emerging studies from our group.

Patricia E. Molina, MD, PhD (She/her)
Richard Ashman, PhD Professor and Chair Department of Physiology
Director Alcohol and Drug Abuse Center of Excellence
LSUHSC New Orleans, LA

Impact of alcohol and aldehyde on innate lung defense: Liver-lung axis.

Todd A. Wyatt, PhD

Description: Alcohol Use Disorders (AUD) contribute to the significant clinical burden of bacterial lung infections. Previously, we established that the lungs of smokers with AUD have malondialdehyde-acetaldehyde (MAA) adducted protein. One of the targets of MAA adduction in lung is surfactant protein D (SPD), resulting in decreased innate cellular defense at the level of both epithelium and macrophages. For SPD to become MAA adducted, threshold concentrations of both acetaldehyde and malondialdehyde must be reached. These aldehydes are typically generated in large amounts by the liver where approximately 85% of consumed alcohol is metabolized by alcohol dehydrogenase (ADH). Only a small amount of CYP2E1 in airway epithelium metabolizes alcohol in the lung, yet the lungs of AUD smokers have the requisite levels of both aldehydes to generate large amounts of MAA adducts. The source of these aldehydes is not fully explained by lung cytochrome action alone. Previously, the liver of alcohol-fed animals and AUD subjects has been shown to generate and release extracellular vesicles (EV) containing ADH as well as reactive oxygen species (ROS)-generating enzymes such as CYP2E1. This raises the intriguing hypothesis that liver-derived exosomes are the source of lung alcohol-metabolizing enzymes that facilitate the heavy reactive aldehyde load observed in lung of those with AUD. Using mouse tracheal epithelial cells and lung organoids exposed to alcohol, we are examining the effect of liver derived EV on MAA adducted SPD formation leading to cilia slowing as an in vitro model of decreased bacterial clearance in AUD lung.

Alcohol-induced gut microbial dysbiosis plays a major role in driving the pathogenic changes in the gut-liver-brain-axis

Shirish Barve, PhD

Description: Emerging evidence has established the important role of the "gut-liver-brain" axis in the development of alcohol-induced hepatic- and neuro- inflammatory changes and injury. Earlier clinical and pre-clinical studies done by others and us using bacterial 16S rRNA gene sequencing have demonstrated that chronic alcohol consumption leads to microbial dysbiosis. However, these studies did not reveal the specific features of gut dysbiosis and consequent functional deficits that are relevant for the pathogenic alterations in the gut-liver-brain axis and development of injury. Our recent work characterizing the gut microbial dysbiosis in animal models of alcoholic liver disease (ALD) and AH patients strongly implicates the decline in butyrate producing communities as a significant feature of alcohol-induced gut dysbiosis. Hence, in relevance to obtaining clinically relevant mechanistic links between intestinal microbial dysbiosis and alcohol associated changes in the gut-liver-brain axis, we have also developed a highly innovative strategy of fecal microbiota transplant (FMT) in conventional mice. Importantly, the data obtained from mice transplanted with fecal microbiome from patients with alcohol-associated hepatitis (AH-FMT), demonstrate that dysbiotic microbiota, marked by a decrease in butyrate producing bacteria, is causally linked to alcohol-induced liver disease and brain inflammation and injury. Notably, data from these studies also support a potential therapeutic role for nutritional interventional strategies to clinically manage gut dysbiosis and alcohol-associated hepatic- and neuro- pathological changes.

Elevated intracellular S-adenosyl-homocysteine: A recurring finding in ALD pathogenesis

Madan Kumar Arumugam[1,2], Srinivas Chava[1,2], Sathish Kumar Perumal[2], Kusum K. Kharbanda[1-3*] ([1]Research Service, Veterans Affairs Nebraska-Western Iowa Health Care System, Omaha, Nebraska, 68105, USA; [2]Department of Internal Medicine and [3]Department of Biochemistry & Molecular Biology, University of Nebraska Medical Center, Omaha, Nebraska, 68198, USA)

Chronic ethanol consumption alters methionine metabolism in many organs including the liver, gut and adipose tissue causing elevations in intracellular S-adenosylhomocysteine (SAH) levels. The rise in intracellular SAH decreases the ratio of S-adenosylmethionine (SAM) to SAH. These metabolic alterations impair the activities of several SAM-dependent methyl-transferases. Because methyltransferases have critical intracellular functions, the downstream consequences of their impaired activities in these three organs perturb the adipose-liver and gut-liver axes to promote the progression of alcohol-associated liver disease.

Alcohol potentiates HIV-induced hepatotoxicity via induction of lysosomal damage in hepatocytes

Moses New-Aaron, Paul Thomes, Murali Ganesan, Terrence M. Donohue, Kusum K. Kharbanda, Larisa I. Poluektova, Natalia A. Osna (University of Nebraska Medical Center and Research Service, Veterans Affairs Nebraska-Western Iowa Health Care System, Omaha, USA)

The incidence of alcohol abuse in people leaving with HIV (PLWH) is twice higher than in general population. Liver injury is important co-morbidity, with mortality rate of 15%. Recently, we have shown that HIV does not replicate in hepatocytes, but exposure to ethanol metabolites like acetaldehyde causes its accumulation in hepatic cells, due to decreased ability of lysosome to degrade HIV proteins, which finally induces apoptosis. In this study, we intend to investigate whether lysosomal damage is related to HIV-acetaldehyde-induced hepatocyte death. The experiments were done on Huh7.5-CYP (RLW) cells exposed to acetaldehyde-generating system (AGS) and

were confirmed by in vivo studies on liver humanized mice treated with HIV and pair-fed control and ethanol diets (10+1 model). We found that exposure to HIV+AGS/ethanol suppressed LAMP1 expression and cathepsin B and L activities and increased co-localization of LAMP1 and GAL3, indicating lysosomal leakage. This leakage led to co-localization of cathepsin B with mitochondrial outer membrane protein TOM 20, which may lead to mitochondrial damage and activation of mitochondrial permeability transition. Furthermore, caspase 3 cleavage under exposure of cells to HIV+AGS was blocked by caspase 9 inhibitor, suggesting that caspase 3 cleavage and subsequent apoptosis can be activated via the intrinsic apoptotic pathway. Apoptotic bodies derived from these HIV - infected cells exposed to AGS after engulfment by hepatic stellate cells and macrophages induced pro-inflammatory and profibrotic cell activation. We conclude that HIV+ethanol metabolism-induced lysosomal damage triggers intrinsic apoptosis in hepatocytes, which causes progression to hepatitis and fibrosis via engulfment of HIV-containing apoptotic bodies by liver non-parenchymal cells.

S 11 YIS 2 Addiction studies/Nordmann Award

Searching for treatment-sensitive neural biomarkers of addiction: is there light at the end of the MRI-tunnel?

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Leptin and ghrelin and a “cross-talk” between both hormones were implicated in the patho-physiology of alcohol dependence, both modulating alcohol craving and drug-seeking. To date, the neurobiological mechanisms underlying those effects are still little-known. We thus investigated the effect of leptin and ghrelin on alcohol cue-induced brain response, alcohol craving and relapse risk in alcohol-dependent subjects.

Seventy abstinent alcohol dependent individuals underwent a functional magnetic resonance imaging (fMRI) alcohol cue-reactivity task and patients` alcohol craving was assessed. Plasma levels of leptin, total and acylated, active ghrelin were measured prior to the fMRI session. Additionally, relapse data was collected during a threemonth follow-up. Associations between hormone levels, mesolimbic cue-reactivity, alcohol craving and relapse risk were tested.

Leptin levels showed a significant negative association to alcohol cue-induced brain response in the striatum and alcohol craving. In addition, there was a significant effect of leptin on time to first heavy relapse in which higher leptin levels predicted longer times to first heavy relapse. Moreover, positive associations between acylated ghrelin and increased cue-reactivity in bilateral insulae as well as increased craving for alcohol during the fMRI task were revealed. Leptin and acylated ghrelin show opposing effects on mesolimbic cue-reactivity and alcohol craving.

We suspect that the reduced striatal cue-reactivity might be the neurobiological correlate of leptin’s effect on relapse-risk. The reported results further support the relevance of appetite regulating hormones in the patho-physiology of addiction and their potential role as future treatment targets.

LRRK2, A PARKINSON'S-ASSOCIATED PROTEIN, REGULATES STRIATAL D1 RECEPTOR FUNCTION AND COMPULSIVE-LIKE ALCOHOL DRINKING IN MICE

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The transition from hedonic to compulsive drinking is a hallmark of alcohol use disorders. Alcohol exerts acute and chronic actions on striatal circuitry and function, which are thought to promote drinking despite negative consequences. Leucine-rich repeat kinase 2 (LRRK2) is a protein with high expression in the striatum which has been shown to regulate dopamine receptor function. Mutations in the Lrrk2 gene are the most common cause of genetic form of Parkinson disease and have been suggested to impair D1 receptor (D1R) internalization and to enhance D1R signaling. We have recently found that mice that naturally develop compulsive alcohol drinking show a dysregulation in the expression of the Lrrk2 gene in the striatum. Here, we test the hypothesis that LRRK2 regulates dopamine receptor function and in doing it so promotes compulsive-like drinking. We found that alcohol drinking decreases LRRK2 phosphorylation and its kinase activity preferentially in the dorsomedial striatum, a region involved in mediating goal directed actions. We generated cell-specific knockout mice and evaluated whether deletion of Lrrk2 gene in different striatal neuron types would affect alcohol consumption. Mice with global deletion of Lrrk2 or with specific deletion in D2R-expressing neurons showed no difference in alcohol drinking compared with littermate controls. However, deletion of Lrrk2 in D1R-expressing neurons (D1-Lrrk2 KO) produced an increase in alcohol consumption relative to littermate control mice. When trained to self-administer alcohol in an operant self-administration task, D1-Lrrk2-KO mice also showed higher responding and intake. In addition, these mice also showed higher breakpoint and drinking was more resistance to shock punishment and taste adulteration with quinine, an indication of higher motivation to drink and drinking despite negative consequences. We further explored possible mechanisms underlying these behaviors and found that D1R function is enhanced in D1-Lrrk2-KO mice. D1-Lrrk2-KO mice showed an upward shift in the dose response curve to the locomotor effects of D1R-like agonist. Moreover, electrophysiological recordings from D1R neurons of D1-Lrrk2-KO mice showed an increase in the excitability of these cells in response to D1R-like agonist compared to D1R neurons of littermate control mice. Additionally, opto-stimulation of D1R neurons terminals in the substantia nigra reticulata in response to D1R-like agonist promoted a larger increase on IPSC in D1-Lrrk2-KO compared to littermate control mice. These findings suggest that loss of Lrrk2 in direct-pathway striatal neurons causes an upregulation of D1R response, both at the cellular and behavioral level, which is conducive to compulsive-like alcohol use.

MEMORY AND SYNAPTIC PLASTICITY IMPAIRMENT AFTER THE VERY FIRST BINGE DRINKING EPISODES IN ADOLESCENT RATS: IS THERE A LINK BETWEEN INFLAMMATION AND GLUTAMATE?

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Keywords: TAK-242, GluN2B, neuroinflammation, LTD, LTP, NMDA

Binge drinking is characterized by excessive consumption of ethanol (EtOH) in a short period of time leading to drunkenness, blackouts or coma. In humans, repeated binge drinking episodes during adolescence impair brain function and learning and memory (Smith et al., 2017; Gierski et al., 2020) while preclinical investigations suggest neuroinflammation as the main neurobiological mechanism underlying such memory impairments after two weeks intermittent ethanol exposure (Pascual et al., 2007). However, less is known about the mechanisms of the very first EtOH exposures, which have been shown to impair learning and

glutamatergic synaptic plasticity in the hippocampus for several days (Silvestre de Ferron et al., 2015; Deschamps et al., 2019; Drissi et al., 2020; Bertrand et al., 2021). These early binges can thus represent gateway to further alcohol abuse and alcohol use disorder. Here, we studied the role of neuroinflammation in learning deficits and in synaptic plasticity impairment in hippocampus slices from male adolescent rats induced by only two binge-like exposures (ethanol 3g/kg, i.p., 9h apart). 48h after the two binges, learning phase in novel object recognition was impaired, long-term synaptic depression in CA1 area of the hippocampus was abolished, long-term synaptic potentiation was increased and NMDA-fEPSPs were more sensitive to a GluN2B subunit antagonist of the NMDA receptor. At the same time point, immunolabelling for Iba1 indicated no microglial reactivity in CA1 and a reduced labelling for neuronal TLR4. No changes were measured in mRNA levels for several inflammatory markers (IL1β, TNF-α, TGFβ and IFN-ϒ). Pretreatment with minocycline or indomethacin, two chemically unrelated antiinflammatory agents or a TLR4 antagonist 30 minutes before each binge, prevented the effects of ethanol at behavioral and cellular level (Bertrand et al., 2021 in revision in ACER; Deschamps et al., submitted to J. Neurosci.; Vilpoux et al., submitted to ACER). Importantly, preliminary results indicate that blocking the GluN2B subunit at 48h delay on slices rescue ethanol-induced LTD abolition; an effect that may have important clinical implications. This work demonstrates that the very first ethanol exposures during adolescence induce transient neuroinflammation and modulate GluN2B functioning to trigger a 48h delayed memory and synaptic plasticity impairments lasting several days. This study further brings interesting view about anti-inflammatory and modulators of the GluN2B subunit that seems good candidates to prevent and to rescue early brain function and memory deficits induced by few binge-drinking episodes during adolescence. An important perspective is now to determine the cellular pathway that relate ethanol-induced neuroinflammation to NMDA receptor functioning after binge drinking.

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Discovery and characterization of ADE and non-ADE rats under longterm alcohol exposure protocol. Biochemical differences in rat brain during alcohol abstinence.

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Alcohol use disorder (AUD) is a cyclical pathology characterized by excessive alcohol consumption with several phases of craving, intoxication, and abstinence1 . Since AUD is a recidivant disorder, to understand the mechanism that underlie relapse is critical to develop new anti-relapse treatments2 . In this sense, understanding the alterations that occur in the brain during abstinence period could also provide relevant information. Nowadays, there exist different preclinical models and paradigms to study relapse behaviour, but some of them rely on one abstinence period although this does not mimic the reality of AUD patients who look for anti-relapse treatments after they experience many periods of alcohol consumption and abstinence3 . Our laboratory has been studying new anti-relapse treatments during many years. For that purpose, we use a model of long-term alcohol consumption in which rats experience several alcohol consumption and deprivation periods. After the forced abstinence, we analyse the alcohol deprivation effect (ADE) that is a transient increase in alcohol consumption after a period of abstinence4 . Besides, to reflect the heterogeneity of alcohol consumer population, we also use non-preferent alcohol rats. By using this model, we have detected two subpopulations of rats: those that usually express ADE phenomenon after abstinence periods, called the "ADE rats" and those that only manifest one or none increase of alcohol consumption during alcohol reintroduction, named "non-ADE rats". To characterise both populations we used 22 male Wistar rats that were exposed during 7 months to four bottles choice paradigm with tap water and ethanol solutions (5%, 10% and 20% v/v on tap water). During this time, they experienced three abstinence periods that were useful to classify each individual rat according to the number of ADE manifestations: two or three for the ADE group and one or none for the non-ADE group. Then, another abstinence and alcohol exposure periods were performed: some animals (n=5) of both groups were sacrificed during abstinence and the rest after 24 hours of alcohol reintroduction. Mass spectrometry analysis was performed to detect alterations in hippocampal oxidative status, and mRNA levels from prefrontal cortex was analysed by RT-PCR to examine expression changes of neuro-inflammatory markers: TNFα, IL6, IL1β, NLRP3, HMGB1, NfκB or iNOS. Statistical analysis of the consumption habits detected differences in either ethanol preferences and ethanol intake (g/kg/day) between the two groups studied. Biochemical results found significant differences in oxidative stress and neuroinflammation levels between ADE and non-ADE rats during abstinence. After obtaining these promising results, we employed this protocol with female Wistar rats who were also divided into ADE and non-ADE groups. Current experiments will clarify if these differences are also found in female subjects.

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IDENTIFICATION AND VALIDATION OF EXPERT PATIENT INTERVENTION AREAS IN ADDICTOLOGY WITH PATIENTS HOSPITALIZED FOR ADDICTION DISORDERS HAPEX-1

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Introduction. The concept of Expert Patients is booming in France(1) in the field of addictions(2), but their roles and impacts on patients have not yet been identified, defined or validated. The objectives of this study are to identify the Expert Patient in Addictology's (EPA) fields of intervention tailored to the specific needs of addicted patients and to develop tools to assess the intermediate effectiveness of the EPA intervention (EPAI).

Method. First part of a mixed qualitative-quantitative study, including patients hospitalized for withdrawal. 1/A qualitative study that elitizes patients' specific expectations and needs regarding EPAI. The verbatims analysis was performed with Nvivo 12.5.0® according to interpretive phenomenological analysis. 2/Translation of the expectations and needs into fields of skills to be developed by the EPA during the intervention with the patient. 3/Literature review for the selection of questionnaires assessing the patient's level of competence following the EPAI, and the development of self-surveys according to the pedagogical methodology.

Results. The qualitative study validated and strengthened the skills already described and identified new ones. A new competency repository has been co-constructed with EPA as a guide for their interventions. It included five major themes: Promote access to care and support for patients in their care trajectory, improve hospital living conditions, encourage patients to express themselves, supporting motivation for change, inform about the disease and its management. To assess these skills, the following three scales were selected: PFQ-2, URICA, and the Rosenberg Self-Esteem Scale; an expert panel developed 2 additional hetero-questionnaires based on the qualitative analysis.

Conclusion. The first part of this study validated and reinforced the fields of competences for the IPEA, and identified new ones, thus allowing the elaboration of a new competence respository. The selected questionnaires, reflecting these skills, will allow in a second part, to evaluate the impact of the IPEA on patients in terms of feasibility, intermediate efficiency and safety through a quasi-experimental comparative study before/after (in process).

Key words: Expert patient, Addictions, Health policy, therapeutic education, mixed qualitative-quantitative method

This study received approval from the CPP - Ile-de-France - VI n° 19.08.05.62342 (ID RCB: 2019-A01661-56).

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A Dominance Analysis Approach Applied to Psychological Factors Associated with Binge Drinking among University Students

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Introduction

Binge Drinking (BD), generally defined as a heavy alcohol consumption over a short period (NIAAA, 2004), is considered as a major public health issue, and especially among university students as it concerns 28% of European student samples (European Union: Directorate General Communication, 2010). While previous studies have evidenced the role of several psychological factors related to BD (e.g., drinking motives[1], drinker identity[2], alcohol subjective norms[3], emotional states[4,5], metacognitions beliefs [6]), little is known about the strength of each of these factors as determinants of BD, mostly because they were often considered separately. The present study therefore proposes to examine the strength of their relative contribution and to identify the most associated factors to BD.

Material and Methods

A community sample of 2851 university students (Mage = 20.50; SD = 2.00; 62.9% of women) participated in an internet survey-based study that was included in the larger research ADUC project ("Alcool et Drogues à l'Université de Caen"). The participants provided information on alcohol related variables (AUDIT, BD score), demographics (sex, age), as well as subjective norm, social identity, external motivations, internal motivations, meta-cognitions, impulsivity and personality traits. The data were first processed via a backward linear regression analysis with repeated K-fold crossvalidation[7] to identify the factors that are significantly associated to BD. Second, to counteract the limitations of traditional statistical methods assessing the strength of factors in models, the relative Abstract submission for young investigator symposia at ESBRA 2021 2 importance of significant psychological factors associated with BD was assessed by performing a bootstrapping dominance analysis[8].

Results

First, the backward linear regression analysis evidenced a best 14-variable model (R^2 = .512, $F(14,1893) = 142$, $p < .001$; $RMSE$ = 14.659; MAE = 10.172), excluding positive metacognitions, negative metacognition (i.e., prejudice), some dimensions of impulsivity (i.e., perseverance and sensation seeking), anxiety trait, and depression. Second, the bootstrapping dominance analysis showed that the strongest variables associated with BD were enhancement motives and drinking identity (average ΔR^2 = 21.81%), followed by alcohol subjective norm and social motives (average ΔR^2 = 13.99%). Other associated variables (average ΔR^2 = 2,84%) were negative metacognition on uncontrollability, sex, coping motives, lack of premeditation, positive metacognition on cognitive self-regulation, positive urgency, lack of perseverance, age, conformity motives and loneliness.

Conclusion

The comparison of the relative importance of variables associated to BD has allowed to identify four major psychological factors, namely enhancement motives, drinking identity, alcohol subjective norm and social motives, suggesting that BD is largely motivated by inter-individual factors. These results have major empirical and clinical implications. Indeed, they highlight specific psychological factors which, not only, should be explored more in depth in the understanding of BD, but also, should be targeted as a priority in new prevention strategies to solve the BD issue. **Keywords:** binge drinking, dominance analysis, identity, enhancement motives, subjective norm, social motives

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ENGAGEMENT OF BRAIN STRESS-REWARD SYSTEMS IN ALCOHOLDEPENDENT MICE AND RATS

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Introduction: Humans with alcohol use disorder often experience negative affect during withdrawal, and depressed mood and anxiety are positively correlated with relapse during abstinence1-2 . The neural adaptations that occur during the transition to dependence are not entirely understood, but may include a gradual recruitment of brain stress circuitry by mesolimbic reward circuitry that is activated during early stages of alcohol use3 . We hypothesized that chronic alcohol increases the activity of a circuit between the ventral tegmental area (VTA) to the central nucleus of the amygdala (CeA), regions important for mediating acute alcohol reinforcement and alcohol withdrawal-associated behaviors, respectively4-6 ; and that activation of this circuit during alcohol withdrawal mediates increases in anxiety-like behavior.

Material and Methods: Adult male C57BL/6J mice and Long-Evans rats were used in all experiments. To characterize CeA-projecting VTA neurons in alcohol-naïve mice and rats, immunohistochemistry or in situ hybridization on retrograde tracer-containing neurons was performed. To evaluate VTA inputs into the CeA, channelrhodopsin (AAV5-hSyn-ChR2-mCherry) was injected into the VTA, and whole-cell recordings were performed in the CeA. Alcohol dependence was induced using a chronic ethanol vapor exposure model. Activity of CeAprojecting VTA rat neurons was measured using retrograde tracing and slice electrophysiology; activation of CeA-projecting VTA rat neurons was measured using retrograde tracing and immune-histochemistry; and the activity of VTA inputs to mouse CeA was measured using opto-genetics and slice electrophysiology. Anxiety-like behavior was assessed in rats during withdrawal. To investigate the mechanism behind altered VTA-CeA physiology in alcohol dependence, we manipulated VTA orexin 1 receptor activity pharmacologically in the above experiments. Results: We demonstrate that the CeA receives input from a mixed population of VTA projection neurons in alcohol-naïve mice and rats. Slice electro-physiology and fos immunehistochemistry were then used to test the effects of alcohol dependence on activity and activation profiles of VTACeA neurons. Our data indicate that alcohol dependence activates midbrain projections to CeA in mice and rats, raising the possibility that this circuit is involved in mediating behaviors (e.g., increased anxiety-like behavior) associated with alcohol dependence. Ongoing experiments explore this possibility by utilizing chemogenetics to investigate the role of the VTA-CeA circuit in anxiety-like behavioral assays in alcohol-dependent and non-dependent rats, as well as pharmacological strategies to investigate the mechanism underlying activation of CeA-projecting VTA neurons. Preliminary data suggest that VTA-CeA neurons may become activated via an orexin receptor-dependent mechanism, and that silencing VTA-CeA projections rescues increased anxiety-like behavior during alcohol withdrawal.

Conclusions: Crosstalk between brain reward and stress systems plays a critical role in behavioral dysregulation induced by alcohol dependence. These studies provide the first in-depth characterization of an understudied VTA-CeA circuit and the first evidence that alcohol dependence activates this circuit in rats and mice. Our data demonstrate a functional connection between VTA and CeA in alcohol-naïve animals. Using anatomical, electro-physiological, and optogenetic approaches, we demonstrate increased activity of the VTA-CeA

circuit in alcoholdependent, withdrawn rats and mice. These data raise the possibility that VTA projections to CeA mediate some aspects of behavioral dysregulation associated with alcohol dependence.

Keywords: Ventral tegmental area, central amygdala, withdrawal, anxiety

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S12 Alcohol effects on brain and treatments

Brain alterations and cognitive deficits induced by alcohol use disorder

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Incredible progress has been made in the last 4 decades in understanding the effects of ethanol on the brain. From a simple molecule that causes non-specific effects on the fluidity of cell membranes, ethanol has been attributed very specific effects at very low doses (ingestion of a standard drink) on a very specific subtype of GABA type A receptors. Ethanol acts on many neurotransmission systems which explains its biphasic effect with first stimulating then depressive effects. Ethanol has rewarding effects relayed by brain mechanisms that are very specific to it compared to other drugs of abuse and explain why alcohol use disorder medications involve the gabaergic, glutamatergic and opioidergic systems. Ethanol has also an impact on the cellular mechanisms underlying learning and memory, the so-called synaptic plasticity phenomenon. Acutely, ethanol binge drinking can induce blackouts. More chronic ethanol intake and repeated binge drinking episodes are known to impair cognitive processes and these effects have been shown to involve neuro-toxicity and neuroinflammation. A continuum of cognitive impairments are well known from light cognitive deficits, Wernicke encephalo-pathy and Korsakoff syndrom including anterograde and retrograde amnesia, executive dysfunction, confabulation, apathy, as well as affective and social-cognitive impairments. Thiamine deficiency is a crucial factor in the etiology of the cognitive deficits. Screening of cognitive impairment is a major challenge in AUD patients seeking treatment, since cognitive dysfunction may impair the overall efficacy of rehabilitation programs and consequently increase relapse rate. Screening tools such as the MoCA (Montreal Cognitive Assessment) and the BEARNI (Brief Evaluation of Alcohol-Related Neuropsycho-logical Impairments) are used in AUD patients.

Medical treatment of alcohol use disorder: A multidisciplinary approach

Julia Sinclair

Comorbidity of alcohol use disorders with substance use disorders and psychiatric disorders

Marcin Wojnar (Department of Psychiatry, Medical University of Warsaw, Warsaw, Poland)

Objectives

Alcohol use disorders are frequent consequences of prolonged and regular use of alcohol. The aim

of the workshop will be presentation of the current knowledge and recommendations in respect of comorbidity with mental and substance use disorders as well as its treatment.

Materials and methods

Available research results published in the current literature were reviewed and analyzed.

Results

General population surveys indicate high prevalence of comorbid psychiatric and substance use disorders. The link between alcohol use disorders and other mental disorders, the so-called "dual disorders", has been increasingly acknowledged. The most common comorbidity includes depressive and anxiety disorders, with increased risk of suicide ideation and attempts, as well as disorders involving substance use and violent or aggressive behavior. The causal pathways between alcohol use disorders and other psychiatric disorders are heterogeneous. Hypotheses explaining these relationships include reciprocal direct causal associations, shared genetic and environmental causes, and shared psychopathological characteristics of broader diagnostic entities. Patients with "dual disorders" show more clinical and social severity and poorer treatment outcomes as compared with patients with only one disorder. Possible factors related to the etiology, course of dual disorders, adherence to treatment, prognosis and risk of relapse remain not fully explained.

Conclusions

During the workshop all these factors will be explored and approaches to treatment of dual disorders' patients will be discussed.

Keywords

Alcohol use disorders, comorbidity, substance use disorders, psychiatric disorders

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Emergency Room: Acute alcohol intoxication and other alcohol-related acute problems (except AWS)

Giovanni Addolorato

S 13 Alcohol-Related liver disease (ALD) – a global epidemic

Pathogenesis and diagnosis of ArLD

Matthew Cramp

Genetic profiling as a tool to advance therapeutic discovery in ArLD

Marsha Morgan

HYPOXIA DETERMINES THE ZONAL-DEPENDENT EXPRESSION OF STARD1 IN ALCOHOLIC LIVER DISEASE

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Alcoholic liver disease (ALD) is a major cause of chronic liver disease and a main reason of liver related death. ALD represents a spectrum of liver alterations that begin with steatosis, which can progress to alcoholic steatohepatitis (ASH) and hepatocellular carcinoma (HCC). Unfortunately, the mechanisms underlying the

progression from steatosis to advanced stages of ALD are still incompletely understood. Besides the abuse of alcohol consumption and genetic determinants, nutritional factors synergize with alcohol to promote ALD progression. Cholesterol metabolism and intracelular trafficking to mitochondria have emerged as important determinants of nonalcoholic steatohepatitis and HCC development. As steroidogenic acute regulatory protein (STARD1) govern the trafficking of cholesterol to mitochondrial inner membrane for metabolism, we examined the regulation of STARD1 in ALD and if its expression occurs in a zonal-dependent fashion. Immunohistochemical analyses of liver sections of patients with ALD or mice fed alcohol revealed a predominant expression of STARD1 in areas stained with glutamine synthase (perivenous zone) but not with CYP2F2 (periportal zone). These findings were confirmed in isolated periportal (PP) and perivenous (PV) hepatocytes from mice fed alcohol. Evidence of alcohol-induced oxidative stress, mitochondrial cholesterol accumulation and mitochondrial dysfunction was predominantly seen in PV hepatocytes, and was ameliorated in PV from *Stard1*$^{\Delta Hep}$ mice fed alcohol. To address the mechanism of STARD1 upregulation in ALD, we addressed the role of hypoxia. Primary mouse hepatocytes cultured under 2% O_2 or cobaltous chloride exhibited increased expression of STARD1. Moreover, mice with hepatocyte-specific *Vhl* knockdown by AAV-TBG-Cre administration to *Vhl*$^{f/f}$ mice, resulted in increased expression of HIF-1□/HIF-2□ and a marked upregulation of STARD1. Consequently, AAV-TBG-Cre-treated *Vhl*$^{f/f}$ mice developed ASH compared to AAV-TBG-GFP-treated *Vhl*$^{f/f}$ mice, and this outcome was ameliorated by simultaneous hepatocyte-specific *Stard1* knockdown (*Vhl*$^{f/f}$/*Stard1*$^{f/f}$ mice treated with AAV-TBG-Cre). Thus, STARD1 is selectively expressed in the perivenous zone in ALD and this event is determined in part through the presence of HRE in the STARD1 promoter.

Big data in ALD: how are multi-omic studies advancing the development of therapeutics for ALD?

Ramon Battaller

S 14 Diagnosis and Interventions in FASD: From Genes to eHealth

Overview of FASD and the work of the Collaborative Initiative on FASD

Edward P. Riley, Ph.D. (San Diego State University, San Diego, CA, USA)

Prenatal alcohol exposure (PAE) can have a devastating impact on the developing fetus, impacting physical and neurological outcomes. Collectively, these effects are termed fetal alcohol spectrum disorders (FASD). The prevalence of FASD in the US was recently reported at 1-5% of the population, with even higher rates in certain countries or among special populations (e.g., some indigenous tribes, people in detention). It has been estimated that more than 630,000 children are born globally each year with FASD (1700/day). Screening, accurate diagnosis, and interventions for FASD are all required to address this major global public health problem. However, there is a mismatch in the number of people impacted by PAE and current diagnostic capacity and this disconnect is most apparent in areas where the rates of FASD are the highest or increasing. WHO reports that 45% of member states have less than 1 physician/1000 people and these are often areas where alcohol consumption is increasing. Missed or misdiagnosis of FASD is common. In one developmental disabilities clinic sample, 86.5% of youth with FASD had never been previously diagnosed or had been misdiagnosed. The Collaborative Initiative on FASD (CIFASD) began almost 20 years ago to address these issues as a multidisciplinary, international consortium aimed at improving the screening, diagnosis, treatment, and understanding of the etiological factors involved in the full range of outcomes resulting from PAE. It encompasses both clinical and basic science studies with an emphasis on the integration of various projects, aimed at addressing the goals of the CIFASD. This Introduction, will briefly present a clinical

profile of FASD and provide a general framework of the research ongoing in CIFASD.

EXPOSURE TO ETHANOL LEADS TO MIDFACIAL HYPOPLASIA IN VANGL2 MUTANTS VIA INDIRECT INTERACTIONS WITH THE SHH PATHWAY

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Introduction

Gene-environment interactions are likely to underlie most human birth defects. Fetal Alcohol Spectrum Disorders (FASD) describes the full range of defects that result from prenatal alcohol exposure. Gene-ethanol interactions underlie susceptibility to FASD but we lack a mechanistic understanding of these interactions. We have previously demonstrated that vangl2 mutants and heterozygotes are sensitized to ethanol-induced midfacial hypoplasia. Here, we used bioinformatic approaches to aid in characterizing the mechanism of the *vangl2*-ethanol interaction.

Material and Methods

Wild-type AB zebrafish and the *vangl2*^{m209} allele were used for all analyses. Mosaicly labeled embryos were generated by injection of EGFP mRNA into a single blastomere at the 32-cell stage. RNA-seq on single embryos was performed by the UT Austin sequencing core. We used the Library of Integrated Network-based Cellular Signatures (LINCS L1000) to identify chemicals that generate similar differential gene expression profiles.

Results

We show that *vangl2* strongly interacts with ethanol during late blastula and early gastrula stages. We performed single-embryo RNA-Seq during early embryonic stages, to assess individual variation in the transcriptional response to ethanol. We used these global changes in gene expression to identify small molecules that would mimic the effects of ethanol via the LINCS L1000 dataset. Surprisingly, this dataset identified the Sonic Hedgehog (Shh) pathway inhibitor, cyclopamine, despite ethanol not altering the expression levels of direct targets of Shh signaling. Indeed, we found that ethanol and cyclopamine strongly interact to disrupt midfacial development. We demonstrate that ethanol synergistically interacts with the loss of *vangl2* to disrupt cell polarity required for convergent extension movements that position a source of Shh critical in midfacial development.

Conclusions

Vangl2 functions as part of a signaling pathway that regulates coordinated cell movements during midfacial development. These movements rely on polarized protrusive activity within migratory cells. Ethanol exposure disrupts this protrusive activity and alters the position of a critical source of Shh signaling that separates the developing eye field into bilateral eyes, allowing the expansion of the midface. Collectively, our results shed light on the mechanism by which the most common teratogen can disrupt development.

Keywords: Wnt/planar cell polarity (PCP) pathway, convergent extension, Vangl2, Sonic Hedgehog (Shh), Fetal alcohol spectrum disorders (FASD), ethanol, cyclopia, polarity, gastrulation

THE ROLE OF SEX AND GENETIC VARIATION IN A MOUSE MODEL OF FAS

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Objectives

Exposure to alcohol during early gestation is associated with craniofacial abnormalities, small eyes, and a wide range of neurological deficits. While genetics are a known mediator of alcohol sensitivity, the role of biological sex in determining the incidence and severity of alcohol-related birth defects is not fully understood. This study compares the effects of gastrulation-stage prenatal alcohol exposure (PAE) in male and female fetuses from several lines of genetically modified mice.

Materials and methods

For all studies, dams were treated with either alcohol (PAE) or vehicle on embryonic day (E) 7.0 and fetuses were observed for craniofacial defects on E17. In addition to C57BL/6J mice, we used mice with gene deletions in either p53

(apoptosis pathway), Htt (intracellular signaling), Kif3a (ciliary transport), or Efcab7 (Smo trafficking) on various background strains.

Results

In C57BL/6J mice, PAE females had a higher incidence of severe eye defects (48%) compared to males (30%). A similar effect was observed in the wild-type mice of all transgenic strains: PAE females had significantly more defects compared to males, independent of background strain. In some strains, an additional gene x sex interaction was observed. In the p53 mice, the protective effect of p53+/- and p53-/- was greatest in males. In the Htt strain, Htt+/- males were protected against a moderate alcohol dose and Htt+/- females were protected against a low alcohol dose. Conversely, Htt+/- females were more sensitive to moderate alcohol, indicating a possible sex x gene x dose interaction. In the Efcab7 or Kif3a mice, PAE females had more eye defects and craniofacial malformations compared to PAE males, regardless of genotype. Kif3a partial deletion did not affect sensitivity to PAE; full deletion of Efcab7 increased the severity of PAE-induced defects in both sexes.

Conclusions

Collectively, these data demonstrate that female mice are more sensitive to prenatal alcohol than males and that sex can interact with certain genotypes to impact outcome. Interestingly, the alcohol exposure in these studies is confined to the period of gastrulation, prior to sexual differentiation. Understanding how early gestational alcohol creates differential long-term outcomes in males and females is an important direction in the field of prenatal alcohol research.

Keywords

Fetal Alcohol Syndrome

UTILIZING 3D FACIAL ANALYSIS TO ESTIMATE THE PREVALENCE OF MINOR FACIAL ANOMALIES IN FASD

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Introduction: The facial gestalt of fetal alcohol syndrome (FAS) has long been established, with diagnosis heavily reliant on identifying the three cardinal features: a smooth philtrum; a thin upper lip; and a reduced palpebral fissure length (PFL). In addition, there are subtle, minor facial anomalies prevalent across the FASD spectrum, which are clinically challenging to distinguish and hence not utilized in clinical assessment. Current facial screening tools available to clinicians provide methods using 2D images require subjective assessment and identify only cardinal features. By employing 3D image analysis, we can objectively identify and measure subtle facial dysmorphism, estimate the prevalence of minor anomalies across the FASD population and provide beneficial clinical feedback on individual facial assessment.

Material and Methods: A Caucasian and African American cohort of participants aged 3 to 18 years was recruited by the CIFASD consortium. Participants underwent full dysmorphology examinations accompanied by facial images acquired using a high-resolution 3D cameras. For each individual, dense surface modelling analysis produced facial heat maps of normalised differences delineating facial dysmorphism. Individual automated anthropometric measurements, and a volumetric measure of the mandible representing micrognathia were calculated to define clinically relevant metrics.

Results: We observe a plethora of facial dysmorphism across the FASD spectrum. In particular, midfacial hypoplasia, micrognathia and shape differences on the nose are observed in a significantly greater number of individuals with alcohol exposure compared to controls. The prevalence of these minor anomalies appear dose dependent in both ethnic groups. The techniques used are capable of detecting subtle facial differences which may provide a robust method for assessing facial dysmorphism in FASD.

Conclusions: Assessment of subtle facial features associated with prenatal alcohol exposure is often overlooked, and is particularly challenging when an individual lacks criteria for a FAS diagnosis. Our

techniques provide a surface-based analysis of facial dysmorphia utilising the precision of 3D imaging. Emerging 3D camera technologies based on smartphone and tablet will reduce the cost and complexity of 3D imaging and hence provide a viable option for future placement in FASD clinics.

Keywords: FASD, Facial Dysmorphism, Prenatal Alcohol Exposure, 3D Facial Analysis

DEVELOPMENT AND EVALUATION OF DIGITAL AND MOBILE HEALTH INTERVENTIONS (eHEALTH) FOR FASD

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Introduction: Fetal alcohol spectrum disorders (FASD) are prevalent conditions worldwide, yet barriers interfere with access to evidence-based care. Mobile health (mHealth) technologies offer promising solutions to overcome some of these barriers.

Material and Methods: We have two current mHealth interventions under development: (1) Families Moving Forward (FMF) Connect for caregivers raising children with FASD (ages 3-12), and (2) My Health Coach for adults with FASD. Our systematic, user-centered design approach to app development and evaluation involves 7 phases. These include: (1) theory guided planning, (2) intervention adaptations for technology, (3) initiate app development, (4) app refinement through stakeholder and focus group input, (5) beta-testing prototypes, (6) feasibility trial and measurement refinement, and (7) randomized controlled trial (RCT) to establish efficacy. FMF Connect will begin phase 7 in the fall and My Health Coach is in phases 3 and 4.

Results: FMF Connect was derived from the therapist-led FMF Program developed by Heather Carmichael Olson and colleagues. FMF content, principles, and methods were successfully adapted for the mHealth format, with the addition of unique content and features. 7 focus groups across 5 U.S. cities were conducted to obtain caregiver input on app interface design and functionalities. Next, two rounds of beta-testing on app prototypes were completed with a total of 45 caregivers and 18 providers. Usage analytics were examined and participant interviews provided important insights into the user experience and ideas for further refinement. A total of 183 caregivers initiated screening for our feasibility trials, with 105 eligible caregivers with complete data. Feasibility trials helped answer important questions about the feasibility of the intervention, trial methods, and user implementation to allow optimization of the upcoming RCT. The My Health Coach app aims to improve the quality of life of adults with FASD and is informed by self-determination theory. It is being developed in partnership with adult leaders with FASD. Their input has been critical in identifying and refining app functionalities and interface design. Focus groups are being initiated.

Conclusions: mHealth interventions offer promise for overcoming barriers to care for FASD across the lifespan.

S 15 Alcohol withdrawal and primary care

Alcohol withdrawal syndrome

Nicola (Nikki) Kalk

Treatments for Adolescent Alcohol Use

Lindsay M. Squeglia, Ph.D. (Medical University of South Carolina, Department of Psychiatry and Behavioral Sciences)

Adolescent alcohol use is pervasive and affects the developing brain. Treatment for adolescence substance use is typically underutilized and/or have only modest effects on reducing use. Utilizing neuroscience in treatment efforts can help make tangible differences in health outcomes by addressing the underlying brain changes involved in substance use disorders. Findings from recent neuroscience-informed adolescent pharmacological and psychosocial substance use treatment interventions will be presented.

Potential New Medications for the treatment of alcohol use disorder and experimental medications

Lorenzo Leggio

Fibrosis screening of ALD based on elastography

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The introduction of elastographic techniques to measure liver stiffness (LS) has significantly improved the fibrosis screening and follow-up of patients with alcoholic liver disease (ALD), one of the major and often overlooked and points in this popular disease. So far, most data have been obtained with transient elastography (Fibroscan, Echosens). In ALD, LS correlates well with histological fibrosis stage with a r>0.7 and AUROCs for F3 and F4 fibrosis are typically higher than 0.9. The confusion about various cut-off values for fibrosis stages is largely due to different distribution and degree of inflammation in the various cohorts. For instance, alcohol withdrawal causes a ca. 20% decrease of LS within one week of alcohol detoxification ultimately leading to a better fibrosis stage in ca. 25%. Ongoing studies suggest that long-term abstinence of more than five years continues to improve LS up to 50%, suggesting partial reversibility of fibrosis/ cirrhosis. So far, levels of AST/GOT are best associated with LS elevation irrespective of fibrosis stage. For these reasons, diagnostic algorithms have been established that either require 1-4 weeks of abstinence or use so called inflammation-adapted cut-off values for improved fibrosis assessment by elastography. Recent unpublished data further suggest that LS is among the best parameters to predict long-term survival in patients with ALD. Taken together, LS measurement has really improved the early diagnosis and follow-up of fibrosis in patients with ALD. There are also preliminary indications that the non-invasive and interactive setting of LS assessment is supporting alcohol withdrawal.

S 16 ALD, NAFLD and bowel disease

Inflammatory bowel disease and alcohol-induced injury

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Introduction: The effects of alcohol misuse in patients with inflammatory bowel disease (IBD) are controversial. The aim of our review is to update the knowledge on the effect of alcohol in patients with ulcerative colitis (UC) and Crohn's disease (CD), related to the daily quantitative alcohol consumption.

Material and methods: A comprehensive literature search and review of 126 articles were retreived on Pubmed with the searching terms: IBD, UC, CD, alcohol.

Results: Alcohol is not considered a risk factor for development of IBD. However heavy drinkers are at high risk for UC (1). Polyphenols and tannins found in wine have beneficial effects on enterocyes. Low quantities of wine are: bactericidal and antiviral. They have probiotic, antioxidant and anti-inflammatory properties, and are reducing the risk of colorectal cancer. In patients with UC, Jiang et al (2) proved the protective effect of drinking (<3days/week) vs. never-drinking subjects. The smokers were not included in the study.

Moderate consumption of red wine for 1 week (1-3 glasses/day, 15 g/day in women and 30 g/day in men) decreased the calprotectine levels. In inactive IBD patients this is considered to predict a low risk of relapse. In active drinkers with CD or UC there is a worsening of symptoms when compared with. IBS patients (3). There was no association between severity of symptoms and quantity of alcohol intake or type of alcohol. High and sustained doses of alcohol (238 g/day) lead to serious mucosal damage, increased risk of colorectal carcinoma (2) and higher incidence of relapses in IBD. Sulfite (in white and red wine, bitter, beer) rather than alcohol itself,

affects UC disease activity; spirits (without sulfite) are not associated with UC activity. In CD patients, consumers of high sugar-alcoholic drinks (smirnof Ice, elefant beer) had higher abdominal pain vs. pure ethanol consumers.

Conclusions: Nonsmoking light drinkers are protected against UC. Heavy drinkers have higher incidence of abdominal pain, risk of relapse and of colon cancer. Moderate alcohol consumption is safe in inactive IBD patients.

Key-words: IBD, alcohol, relapse, calprotectine

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Non-alcoholic steatohepatitis and the microbiome

Steve Malnick

Dysfunction of Intestinal Antimicrobial Peptides in the Pathogenesis of Alcoholic Hepatitis

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Background & Aims: Microbial dysbiosis is associated with alcoholic hepatitis (AH) with the mechanisms yet to be elucidated. Intestinal antimicrobial peptides (AMPs) play a critical role in regulating microbiota homeostasis and limiting bacterial translocation. Among these AMPs, α-defensins, that are secreted by Paneth cells, account for 70% of total bactericidal activity in the small intestine. The present study aimed to determine the effects of alcohol on intestinal AMPs, the link between PC dysfunction and the development of AH, and the underlying mechanisms of alcohol-induced intestinal AMPs dysfunction.

Methods: Translocation of pathogen-associated molecular patterns (PAMPs) was determined in patients with severe AH and in a mouse model of alcohol-related liver disease. Microbial composition and PC function were examined in mice. The link between α-defensin dysfunction and AH was investigated in α-defensin deficient mice. Synthetic human α-defensin 5 (HD5) was orally given to the alcohol-fed mice to test the therapeutic potential. The role of zinc deficiency in α-defensin was evaluated in acute and chronic mouse models of zinc deprivation and in a mouse model of PC-specific deletion of a zinc transporter, ZIP8.

Results: Hepatic inflammation was associated with PAMP translocation, and lipocalin-2 (LCN2) and CXCL1 elevation in AH patients. Antibiotic treatment, lipopolysaccharide injection to mice, and *in vitro* experiments showed that PAMPs, but not alcohol, directly induced LCN2 and CXCL1. Chronic alcohol feeding caused systemic dysbiosis and PC α-defensin reduction in mice. Knockout of functional α-defensins synergistically affected alcohol-perturbed bacterial composition and gut barrier, and exacerbated PAMP translocation and liver damage. Administration of HD5 effectively attenuated cecal microbial dysbiosis, especially increased *Akkermansia muciniphila*, and reversed alcohol-induced deleterious effects. Zinc regulated PC homeostasis and α-defensins function at multiple levels, and dietary zinc deficiency exaggerated the deleterious effect of alcohol on PC bactericidal activity. Deletion of PC ZIP8 further reduced PC zinc levels, impaired bacterial killing, and promoted ALD in mice.

Conclusions: Taken together, the study suggests that alcohol-induced PC α-defensin dysfunction is mediated by zinc deficiency and involved in the pathogenesis of AH. HD5 administration may represent a novel and promising therapeutic approach for treating AH.

H_2O_2-mediated autophagy during ethanol metabolism

Cheng Chen, Shijin Wang, Johannes Mueller, and Sebastian Mueller* (Center for Alcohol Research and Salem Medical Center, University of Heidelberg, Heidelberg, Germany)

ABSTRACT:

Background: Alcoholic liver disease (ALD) is the most common liver disease worldwide and its underlying molecular mechanisms are still poorly understood. Moreover, conflicting data have been reported on potentially protective autophagy, the exact role of ethanol-metabolizing enzymes and ROS.

Methods: Expression of LC3B, CYP2E1, and NOX4 was studied in a mouse model of acute

ethanol exposure by immunoblotting and immunohistochemistry. Autophagy was further studied in primary mouse hepatocytes and huh7 cells in response to ethanol and its major intermediator acetaldehyde. Experiments were carried out in cells overexpressing CYP2E1 and knock down of NOX4 using siRNA. The response to external H_2O_2 was studied by using the GOX/CAT system. Autophagic flux was monitored using the mRFP-GFP-LC3 plasmid, while rapamycin and chloroquine served as positive and negative controls.

Results: Acute ethanol exposure of mice over 24 hours significantly induced autophagy as measured by LC3B expression but also induced the ROS-generating CYP2E1 and NOX4 enzymes. Notably, ethanol but not its downstream metabolite acetaldehyde induced autophagy in primary mouse hepatocytes. In contrast, autophagy could only be induced in huh7 cells in the presence of overexpressed CYP2E1. In addition, overexpression of NOX4 also significantly increased autophagy, which could be blocked by siRNA mediated knock down. The antioxidant N-acetylcysteine (NAC) also efficiently blocked CYP2E1- and NOX4-mediated induction of autophagy. Finally, specific and non-toxic production of H_2O_2 by the GOX/CAT system as evidenced by elevated peroxiredoxin (Prx-2) also induced LC3B which was efficiently blocked by NAC. H_2O_2 strongly increased the autophagic flux as measured by mRFP-GFP-LC3 plasmid

Conclusion: We here provide evidence that short-term ethanol exposure induces autophagy in hepatocytes both *in vivo* and *in vitro* through the generation of ROS. These data suggest that suppression of autophagy by ethanol is most likely due to longer alcohol exposure during chronic alcohol consumption with the accumulation of e.g. misfolded proteins.

Keywords: Alcohol liver disease (ALD); Ethanol metabolism, Cytochrome P450 2E1(CYP2E1), NADPH oxidase (NOX), Hydrogen Peroxide (H_2O_2), Reactive Oxygen Species (ROS).

Non-invasive Biomarkers of Liver Inflammation and Cell Death in Response to Alcohol Detoxification

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Introduction: Alcohol-related liver disease (ALD) represents the most common liver disease worldwide, however, the underlying molecular mechanisms are still poorly understood. Namely centrilobular inflammation and programmed cell death are characteristic to ALD and it remains to be elucidated why they persist despite the absence of alcohol.

Aims: To study the effects of alcohol withdrawal in a cohort of heavy drinkers and the role of cirrhosis by using non-invasive biomarkers such as cytokines, apoptotic and angiogenic markers.

Methods: Caspase 3-cleaved M30, M65, cytokines (IL-6, IL-8), tumor necrosis factor alpha (TNF-α), transforming growth factor (TGF-β) and vascular endothelial growth factor (VEGF) were measured in 114 heavy drinkers. The role of alcohol detoxification was investigated in 45 patients. The liver histology was available in 23 patients. Fibrosis stage and steatosis were assessed by measuring liver stiffness (LS) and controlled attenuation parameter (CAP) in all patients using transient elastography (FibroScan, Echosens, Paris). Mean observation interval between the measurements was 5.7 ± 1.4 days (mean + –SD).

Results: Patients consumed a mean of 204 ± 148 g/day alcohol with a heavy drinking duration of 15.3 ± 11.0 years. Mean LS was 20.7 ± 24.4 kPa and mean CAP was 303 ± 51 dB/m. Fibrosis distribution was F0–38.1%, F1-2–31%, F3–7.1 and F4–23.9%. Apoptotic markers M30 and M65 were almost five times above normal. In contrast, TNF- α a, IL-8 and VEGF were only slightly elevated. Patients with manifest liver cirrhosis (F4) had significantly higher levels of M30, M65, IL-6 and IL-8. Histology features such as hepatocyte

ballooning, Mallory-Denk bodies, inflammation and fibrosis were all significantly associated with elevated LS, and serum levels of TNF-alpha, M30 and M65 but not with CAP and other cytokines. During alcohol detoxification, LS, transaminases, TGF- β, IL-6, IL-8 and VEGF decreased significantly. In contrast, no significant changes were observed for M30, M65 and TNF- α and M30 even increased during detoxification in non-cirrhotic patients. Profibrogenic cytokine TGF-beta and pro-angiogenic cytokine VEGF showed a delayed decrease in patients with manifest cirrhosis.

Conclusion: Patients with alcohol-related cirrhosis have a pronounced apoptotic activity and a distinct inflammatory response that only partly improves after 1 week of alcohol detoxification. Alcohol withdrawal may represent an important approach to better dissect the underlying mechanisms in the setting of alcohol metabolism.

Chaperone-Mediated Autophagy Substrate Stabilization Potentiates EtOH-Induced Lipogenesis in Liver Cells

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Background: Alcohol-induced fatty liver is characterized by greater numbers and sizes of lipid droplets (LD) in hepatocytes. LDs are enclosed by a phospholipid monolayer, which harbors the LD proteins that regulate triglyceride (TG) metabolism, LD trafficking, and LD fusion with other organelles. We recently reported that chronic EtOH administration significantly alters the LD membrane-associated proteome, enriching lipid biosynthetic proteins and reducing lipid degrading proteins, thereby causing expansion of LD numbers and sizes in liver cells. We hypothesized that chronic EtOH administration disrupts the degradation of lipogenic proteins by lysosomes, thereby, causing their stabilization and enhancing their participation in *de novo* lipogenesis. Here we sought to determine whether chronic EtOH feeding interferes with chaperone-mediated autophagy (CMA) to promote hepatic LD accumulation.

Methods: *In vivo* studies used mice or rats' pair-fed liquid control or EtOH diet for 5-9 wk. Liver LDs were purified by gradient centrifugation. LD membrane proteins were subjected to MS-proteomics to identify LD proteome. Cell culture experiments used EtOH-metabolizing, HepG2-derived VA-13 cells.

Results: In silico screening, using KFERQ motif finder of MS-proteomics dataset identified 39 LD membrane proteins that carry the KFERQ pentapeptide motif that is essential for HSC-70 (chaperone) targeting of proteins for degradation in lysosomes. Among these KFERQ motif containing proteins, chronic EtOH feeding elevated the levels of acetyl-CoA carboxylase (ACC) and fatty acid synthase (FASN), which catalyze de novo lipogenesis, Hydroxysteroid 17β-dehydrogenase 13, which enhances lipid accumulation and perilipin-3 (PLIN-3) which renders LDs resistant to lipolysis and lipophagy. Immunoblotting of liver homogenates and LD fractions confirmed that EtOH feeding upregulated all the aforementioned proteins. In VA-13 cells, transient overexpression of LAMP2A decreased cellular triglycerides (TG) and blocked TG accumulation after EtOH exposure. LAMP2A over-expressing cells also exhibited reduced levels of ACC, FASN and PLIN-3. Compared with pair-fed controls, lysosome-enriched fractions from EtOH-fed mice exhibited significantly lower levels of LAMP2A and HSC-70, both parts of the CMA machinery. Moreover, lysosomes purified from EtOH-fed mice exhibited a 20% lower ability to degrade KFERQ-containing ^{125}I-ribonuclease *in vitro*.

Conclusion: EtOH feeding compromises the CMA degradation machinery, causing stabilization of CMA target proteins that enhance lipid biosynthesis, and therefore, potentiate development of alcohol-induced fatty liver.

Structural mapping and higher brain function

Phillippe de Timary

Altered diffusivity in the alcoholic brain – what are the functional consequences

Santiago Canals

Abstract: Already moderate alcohol consumption has detrimental long-term effects on brain function. However, how alcohol produces its potent addictive effects despite being a weak reinforcer is a poorly understood conundrum that likely hampers the development of successful interventions to limit heavy drinking. In this talk, I will present our recent published and unpublished results showing widespread increased mean diffusivity in the brain grey and white matters of chronically drinking humans and rats. These alterations appear soon after drinking initiation in rats and persist (grey matter) or even progress (white matter) into early abstinence in both species. Using two rat models of alcohol use disorders we associated grey matter changes with a robust decrease in extracellular space tortuosity explained by a microglial reaction, and white matter alterations with a reduced content in myelin basic protein, suggesting demyelination. Using computational models that incorporate the experimentally found alterations, we postulate important effects on dopamine neurotransmission and memory formation.

Probing functional brain networks in alcoholism by using advanced signal processing techniques and machine learning

Raul Muresan

Using machine learning, structural differences in adolescent brains can predict alcohol misuse in the future (previous: Machine learning-based decoding of binge drinking and the challenges)

Roshan Rane

Abstract: Excessive and risky alcohol consumption during adolescence is a risk factor that can lead to alcohol addiction later in life. Adolescence is also a period of significant brain development and reorganization. We analyzed structural imaging data (T1-weighted and DTI) in the IMAGEN dataset (n~1182) from three time points of adolescence (ages 14, 19, and 22) with a goal to understand how the adolescent brain structures and adolescent alcohol misuse (AAM) are related. Machine learning models were used to predict AAM by the age of 22 using ten different alcohol misuse phenotypes such as the amount of alcohol consumed, frequency of alcohol use, binge drinking, age of onset, and their combinations and longitudinal trajectories. After systematically evaluating four machine learning models, multiple confound-control techniques, and different phenotypes of alcohol misuse, we found that alcohol misuse at age 22 was significantly predictable from their brain structure from as early as age 14. Between the ten AAM phenotypes, binge drinking was the most predictable phenotype, in contrast to frequency, amount, or even the AUDIT scores. With the best setting, AAM at age 22 could be predicted with a balanced accuracy of \$72\%\$, \$76\%\$, and \$77\%\$ from brain structure at ages 14, 19, and 22, respectively. Our results not only show that structural differences in the adolescent brain can significantly predict alcohol misuse, but also that these differences might precede alcohol misuse behavior. Furthermore, we also show that the choice of AAM phenotype used as the label, the machine learning model, and the confound control techniques, are of crucial choice for multivariate studies such as machine learning when analyzing psychiatric disorders with weak effect sizes.

Acute liver failure due to alcohol intoxication- therapeutic options

Carmen Fierbinteanu-Braticevici („Carol Davila" University of Medicine and Pharmacy Bucharest, Department of Gastroenterology, University Hospital Bucharest)

The spectrum of alcohol-related liver diseases (ALD) includes many entities: steatosis, steatohepatitis, progressive liver fibrosis, and cirrhosis. Active alcohol consumption can induce alcoholic hepatitis and usually is the most frequent event precipitating the development of Acute-on-chronic liver failure (ACLF) in the context of ALD (ALD-ACLF). Acute liver failure is relatively common and severe complication of alcoholic hepatitis (AH) and is characterised by liver cell dysfunction leading to coagulopathy and hepatic encephalopathy, after binge drinking. Severe alcoholic hepatitis (AH) carries a very high short-term mortality and the strongest predictive factor is hepatic encephalopathy.

ACLF is characterised by acute deterioration, organ failures, evidence of systemic and hepatic inflammation and a high risk of mortality.

Severe alcoholic hepatitis and ACLF need immediate referral to hospital for assessment and support and the treatment is essential. Patients with severe alcoholic hepatitis may benefit from specific therapies directed toward reducing liver injury and suppressing inflammation. Traditional approaches focuse on nutritional support and corticosteroids may be considered, although evidence for their effectiveness remains inconclusive. Nutritional support may be required to maintain muscle mass and prevent catabolism. Multivitamins and supplements to correct specific deficiencies are also commonly used in clinical practice. Vitamin B1 (thiamine) supplementation is recommended to prevent neurological complications like Wernicke-Korsakoff's syndrome. Because glucocorticoids are marginally effective there is an unmet need for newer therapies. The addition of intravenous N-acetyl cysteine (NAC) to prednisolone may improve the 30-day survival of patients with severe AH. Various other treatments remain experimental. Several studies have demonstrated the efficacy of Granulocyte - Colony Stimulating Factor in mobilizing stem cells from bone marrow to liver, with resultant improvement in clinical, biochemical, and histological profile in patients with liver failure. Some pilot studies suggest that healthy donor faecal microbiota transplantation has a beneficial effect on the outcome of patients with alcohol related ACLF. In selected cases, the treatment consists in the use of liver-assist devices (artificial livers) with transient improvement of liver failure. Other investigators have proposed pilot studies to determine whether liver transplantation improves the survival of patients with severe alcoholic hepatitis.

Severe acute ethanolic intoxication-management of critical ill patient

Ovidiu Bedreag

COAGULATION DISORDERS IN PATIENTS WITH ALCOHOLIC LIVER CIRRHOSIS. WHAT EVERY PHYSICIAN NEEDS TO KNOW?

Liana Gheorghe, MD, PhD (Professor of Gastroenterology & Hepatology *Carol* Davila University of Medicine and Pharmacy Center for Digestive Diseases and Liver Transplantation Fundeni Clinical Institute Bucharest, Romania

ABSTRACT

It is now recognized that in liver cirrhosis, irrespective of etiology, the intact hemostatic competence is replaced by a re-balanced low-level hemostatic equilibrium due to a concordant reduction in pro- and anti-coagulant factors. Re-balanced hemostasis results in complex alterations of all 3 phases of hemostasis: primary hemostasis, coagulation, and fibrinolysis. Alcoholic etiology can additionally affect coagulation; however, it also may have bidirectional effects that balance one another. Specifically, alcohol can impair platelet production and initial clot formation but can also decrease fibrinolysis.

Conventional coagulation tests do not fully reflect the abnormalities of hemostasis and do not accurately predict the risk of bleeding.

Commercially available global coagulation tests, collectively known as "viscoelastic tests", including thromboelastography [TEG], rotational thromboelastometry [ROTEM], and sono-rheometry [SONOCLOT] analyze all components of hemostasis including the dynamics of clot formation, clot strength, and clot stability and represent a promising point-of-care tool for assessing bleeding risk and guiding therapy in patients with liver cirrhosis and coagulopathy.

Rebalanced hemostasis in liver disease is labile and may be tipped toward hemorrhage or thrombosis by various triggers such as systemic infection, volume status, variceal bleeding, decompensated liver cirrhosis, invasive procedures, or inadequate hemotherapy with prothrombotic components. The common clinical scenarios associated with cirrhotic coagulopathy are bleeding scenario (phenotype) and thrombotic scenario (phenotype).

In the setting of bleeding, blood products transfusions should be used judiciously because they increase portal pressure and carry a risk of transfusion-associated circulatory overload, transfusion-related acute lung injury, infection transmission, alloimmune-zation, and/or transfusion reactions. The following transfusion thresholds for management of active bleeding or high-risk procedures may optimize clot formation in advanced liver disease: hematocrit ≥25%, platelet count >50,000/mmc, and fibrinogen >120 mg/dL.

In the setting of thrombotic complications, systemic heparin infusion is recommended for symptomatic deep vein thrombosis, thromboembolism and portal and mesenteric vein thrombosis. Treatment of incidental portal and mesenteric vein thrombosis depends on estimated impact on transplantation surgical complexity vs risks of bleeding. Therapy with low-molecular-weight heparin, vitamin K antagonists, and direct-acting oral anticoagulants improve portal vein re-permeabilization vs. observation alone.

Hepato-renal syndrome in patients with acute alcoholic intoxication

Anca Trifan

Particularities of intensive care in patients with alcohol use disorder

Dorel Sandesc

S 19 Alcohol and metabolic syndrome

Alcohol and metabolic syndrome: clinical aspects

Philippe Mathurin

Alcohol, metabolic syndrome and the liver: epidemiology

Helena Cortez-Pinto (Clínica Universitária de Gastrenterologia, Laboratório de Nutrição, Faculdade de Medicina, Universidade de Lisboa, Portugal)

Alcohol and obesity are the two main causes of CLD in Western countries and have increased significantly in the last decades. Both are predicted to further increase in next years if no effective policy measures are taken. There is also evidence of an interaction of the two factors, alcohol and obesity, the latter often associated with the metabolic syndrome, in causing liver disease worldwide, from which we will show evidence. In this presentation we will present the recent epidemiological data regarding alcohol-related liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD) worldwide. It will also be discussed the predictions regarding the two main risk factor's progression, obesity and alcohol, and the effect it will have in liver disease prevalence. We will also present what are the main policies that have been considered effective and that relate mostly with taxation. In the case of alcohol, this can be done through policies such as the Minimum Unit pricing (MUP), where taxation defines a floor price beneath which alcohol cannot be sold, based on the amount of pure alcohol in a product, or other taxation measures, as well as several other measures related with advertising and marketing. In the case of obesity, and risk factors for the metabolic syndrome, it is again taxation, in this case, mostly for sugar and

sweetened products (SSB). Preliminary data from EASL Hepahealth II project, using a microsimulation model, will be presented, showing the predicted effect of reduction of alcohol consumption and BMI in liver disease. We will also briefly discuss the effect of COVID-19, regarding alcohol consumption and obesity.

HISTOLOGICAL LESIONS CAN PREDICT RESPONSE TO CORTICOSTEROIDS IN PATIENTS WITH SEVERE ALCOHOL-RELATED STEATOHEPATITIS (ASH).

Carolin Lackner

Screening for NAFLD and ALD in patients with type 2 diabetes and in general population

Hannes Hagström

S 20 YIS3 addiction studies

More than just an alcoholic person: Preventing stigma and dehumanization with multiple categorization

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Introduction. People with alcohol use disorder (AUD) are suffering from social stigma and may even be denied a full human status.1,2 These social misperceptions have detrimental outcomes, discouraging people to seek care for their disease.3 Recent research suggests that the dehumanization process of people with AUD could be prevented by the use of a multiple categorization strategy (i.e., a strategy in which stigmatized individuals are presented with multiple categories including the stigmatizing one).4 The present research aims at specifying conditions that may improve the multiple categorization strategy efficiency in preventing AUD social stigma. First, a pilot study (PS) was conducted in order to compare the benefit of multiple categorization strategy over the individuation approach. 5 Second, two pre-registered studies (PRS) were conducted. The first one (PRS1) aimed to determine the optimal number of categories to present. In complement, the second one (PRS2) intended to clarify the impact of "other" categories' valence.

Methods. These studies were conducted among University Students (NPS = 187, Mage= 19.95, SDage = 1.24; NPRS1 = 232, Mage= 20.76, SDage = 3.86; NPRS2 = 228, Mage= 20.66, SDage = 3.22). Three randomized controlled studies were conducted. Systematically participants had to form an impression about targets with AUD that were presented either only through the "alcoholic person" stigmatizing category (i.e., control condition which was constant across studies) or through their membership to multiple categories (three experimental conditions for each study), which varied depending on the aim of the study. In the pilot study, targets with AUD in experimental conditions were presented with either five categories (e.g., a sportsman), with five individuating information (e.g., he enjoys sports), or a five combination of both. In PRS1, they were presented with either three, five, or seven neutral categories. In PRS2, they were presented with either five negative, neutral, or positive categories (valence was pre-tested in an independent study). Several approaches were combined to investigate humanity attributions to people with AUD. Specifically, scales of competence and warmth attributions, scales of uniquely human and human nature traits attributions, and scales of agentism and experience attributions were used. 6–8

Results. The pilot study evidenced the beneficial impacts of the multiple categorization strategy (Fs > 5.52, ps < .05, η 2

ps > .02), but not consistently those of an individuation strategy. PRS1 revealed the beneficial impact of presenting the AUD target through three categories, which was reinforced with five categories (Fs > 4.82, ps < .01, η 2 ps > .06). PRS2 revealed that the multiple categorization strategy works even if the AUD target is presented only with negatively valenced categories, but mostly works better when presented with neutral or positive categories (Fs > 3.68, ps < .05, η 2 ps > .05).

Conclusion. Providing cares for people with AUD needs them to recognize their disorder, while avoiding its stigma. The multiple categorization may constitute a key issue to address this contradictory injunction for people with AUD. Implications of these findings for the practitioner will be discussed and propositions will be made to translate these findings in the applied field.

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Epigenetic mechanisms underlying alcohol use and anxiety disorders

Estelle Barbier

A major challenge to addressing the treatment needs of patients with AUD is the high prevalence of co-occurring psychiatric disorders, of which anxiety disorders are the most common. Both AUD and anxiety disorders are characterized by broad changes in gene expression within brain regions that include the prelimbic cortex (PL) and the amygdala complex. In rats with a history of alcohol-dependence, we identified prelimbic DNA hypermethylation as a mechanism through which chronic intermittent alcohol exposure increases alcohol consumption. Inhibition of DNA methyltransferase 1 (DNMT1) partially rescued this behavior and also restored alcohol-induced gene expression changes in the PL, including a downregulation of the histone methyltransferase Prdm2. Knock-down of Prdm2 in the PL of naïve animals was sufficient to promote alcohol-related behaviors. In particular, it increased sensitivity to stress-induced reinstatement by foot shock, suggesting a link between PRMD2 and stress responses. Recent data show that PRDM2 also contributes to behaviors characteristic of anxiety disorders, such as excessive fear. In a model of cued conditioned fear, PL Prdm2 knock-down persistently increased the expression of fear, without affecting acquisition or retrieval processes, suggesting that it affects the consolidation of the fear memory. Selective knock-down in neurons projecting from the PL to the basolateral amygdala (BLA) was sufficient to promote the increased fear expression. RNA sequencing specifically in PL-BLA projecting neurons, using viral translating ribosome affinity purification (vTRAP), showed a marked upregulation of genes involved in synaptogenesis. Preliminary GCaMP fiber photometry and patch-clamp electrophysiology data show an increased neuronal activity in the BLA. This suggests that PRDM2 regulates fear expression by modulating fear memory consolidation, and specifically strengthening this process by promoting synaptogenesis in the PL-BLA circuit. Together, our findings highlight the contribution of epigenetic

mechanisms in mediating the behavioral consequences of alcohol-dependence, including anxiety-like behaviors.

Early recovery of neuropsychological impairments during detoxification in patients with alcohol use disorder

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Background. Thirty to 80% of patients with alcohol use disorder (AUD) present neuropsychological impairments resulting in impairments on their autonomy. Only a few studies have assessed early recovery of cognitive disorders with standardized and simple tools designed for clinical practice such as The Brief Evaluation of Alcohol-Related Neuropsychological Impairment (BEARNI). The objective of this study was to assess recovery of alcohol-related neuropsychological deficits during a detoxification program using the BEARNI tool**.**

Methods: Thirty-two patients with AUD using DSM-V criteria (24 men, mean age = 45.5 ±6.8 years old) and 32 healthy control subjects (21 men, mean age = 46.5 ± 5.7 years old) were assessed using the BEARNI. Patients with AUD were assessed 8 ± 2 days on average after alcohol cessation and then were reassessed within 18 ± 2 days after alcohol cessation (T2). The primary study endpoint was the proportion of patients initially impaired at T1 who recovered cognitive functions at T2 assessment.

Results. At T1, 19 (59.3%) patients with AUD were impaired had at least one cognitive function assessed by the BEARNI. At T2 we found a significant recovery of cognitive deficit. 11 (58%) patients with cognitive impairments at T1 had normal scores at T2 (chi-2=7.7, p=0.005). In the AUD sample, the BEARNI total and cognitive scores signify-cantly increased from T1 to T2 ($F=14.7$, $p = 0.0002$ and $F=14.8$, $p < 0.0003$, respectively). All subtest scores significantly increased between T1 and T2 (ie. the delayed free recall, the alternating verbal fluency, visuospatial abilities and alphabetical ordination (respectively RM ANOVA; $F(1, 62)=8.2$, $p=0.006$); $F(1, 62)=9.6$, $p=0.003$); ($F(1, 62)=10.0$, $p=0.002$); $F(1, 62)= 14.7$, $p=0.0002$).

Conclusions. 58 % of patients with AUD included in the present study recovered within 18 days of abstinence. Medical management of AUD before 18 days of abstinence could be more intensive.

Keywords: neuropsychological assessment, BEARNI, alcohol use disorder, cognitive impairments, recovery.

ADVERSE CHILDHOOD EXPERIENCES (ACES) IN YOUNG CHILDREN WITH FETAL ALCOHOL SPECTRUM DISORDER (FASD) AND EFFECT ON BEHAVIOR PROBLEMS

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Introduction: People with fetal alcohol spectrum disorders (FASD) are an under-recognized, underserved population who often experience significant challenges. FASD refers to a number of neurodevelopmental and physical differences associated with prenatal alcohol exposure (PAE). Only recently has research been formally conducted on adverse childhood experiences (ACEs) in people with FASD despite highly elevated rates (Kambeitz et al., 2019; Flannigan et al., 2021). Given that ACEs predict risk for physical, mental, and

emotional health issues later in life (Felitti et al., 1998), research in this area is essential to inform intervention work. The current study aimed to: 1) describe ACEs in young children with FASD; and 2) examine how ACEs, child age, and family structure are related to child behavior problems.

Methods: Participants were a convenience sample of 87 caregivers of children with FASD enrolled in a feasibility study for a mobile health parenting intervention. Children had a mean age of 8.18 years (SD=2.62) and had a diagnosed FASD or documented PAE. At baseline, caregivers completed a demographic survey and measures of child and family functioning. ACEs were measured using the ACEs Questionnaire (Felitti et al., 1998) and behavior problems were measured using Intensity T-scores from the Eyberg Child Behavior Inventory (ECBI; Eyberg & Pincus, 1999). Data were analyzed using Pearson correlations and linear regression.

Results: On average, caregivers endorsed 3.10 (SD=2.99) ACEs. The most frequently endorsed ACE risk factor was having lived with a household member who was depressed or mentally ill (N=51, 60.0%), closely followed by having lived with someone who was addicted to alcohol or used street drugs (N=45, 52.9%). Experiencing sexual abuse was least frequently endorsed (N=9, 12.9%). Correlational analyses revealed that current foster care placement was significantly associated with a higher total ACEs score (r=.25, p<.05). Total ACEs score was not significantly associated with child age, household income, household size, or caregiver type other than foster parent.

Mean ECBI Intensity T-score was 69.94 (SD=9.17). Scores ranged from 46-88, with a striking 84.88% of the sample scoring at or above the clinical cut-off of 60. To examine how ACEs are related to child behavior problems, linear regression was conducted in which total ACEs score predicted intensity of child behavior on the ECBI, controlling for child age, household size, caregiver type, and household income. Overall ACEs significantly predicted child behavior problems (β=.33, p<.01). No other variable significantly predicted child problem behavior.

Discussion: Children with FASD or PAE had an ACEs score of 3.10 on average, almost twice that of the general population (1.56; Giano et al., 2020). Children with higher ACEs had greater problem behavior reported by parents on the ECBI. This underscores the importance of screening for ACEs and providing appropriate support for children with FASD or PAE who have experienced adverse childhood events, especially those in foster care. Findings strongly emphasize the need for trauma-informed clinical care for children with FASD or PAE— and increased accessibility of care. Future research should examine potential mechanisms for how ACEs and later behavior problems relate to optimally inform intervention.

Keywords: fetal alcohol spectrum disorders; prenatal alcohol exposure; FASD; adverse childhood experiences; foster care; intervention;

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PILOT STUDY CANNAVAP: EFFICIENCY OF CANNABIDIOL INHALED WITH A VAPING DEVICE IN CANNABIS REDUCTION OR WITHDRAWAL

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The study protocol was validated by the person's protection comitte Sud Ouest et Outre mer the 21st of septembre 2020 (CPP 1-19-041/ ID 3012).

Introduction

Cannabis is the most used illegal substance in the world(1). There are currently no specific pharmacotherapies approved for cannabis use disorder (CUD) (2,3). Cannabidiol (CBD) is the second most present cannabinoid in cannabis(4). It is already used as a treatment in epilepsy(5), and considered to be safe, with few adverse effects, and without risk of addiction(6–8). In preclinical stydies, CBD reduced drug seeking behaviors for various substances(9). Two case reports(10,11) and one clinical study(12) suggest a potential of CBD for the treatment of CUD. In many countries, CBD is available as a consumer good, especially in liquid for vaping devices. In everyday clinical practice, patient willing to quit or reduce their cannabis consumption are already experimenting with CBD. The aim of our study was to evaluate the potential of CBD inhaled via a vaping device in CUD.

Material and Methods

We conducted an exploratory, observational, non-randomized, open-label study in a lowthreshold addiction medicine facility in Paris. Participants were adult volonteers with CUD only. The primary endpoint was the reduction of 50% or more of the declared number of joints smoked per day at the end of the 3 months follow up. Participants were given a vaping device. Patients were evaluated every week (drug consumption, craving, withdrawal symptoms, urine drug testing, expired air carbon monoxide testing). They were given a refill of liquid with CBD in one of 3 concentrations (33,3mg/ml, 66,6 mg/ml or 100 mg/ml).

Results

Between october 2020 and february 2021, 22 patients were included. In march 2021, 8 patients were still undergoing trial (end of the follow up in may 2021), 7 had been lost to follow up, and 7 had completed the study. Out of these 7 participants, 4 had reduced their cannabis consumption by at least 50%. No patient reported difficulties to use the vaping device.

Conclusions

Our preliminary results regarding both the feasibility of this type of study and the potential of CBD in CUD are encouraging. The purpose of this pilot study is to evaluate the possibility of setting up a more ambitious, randomized, double blind versus placebo clinical trial.

Keywords

Cannabis use disorder. Cannabidiol. Clinical trial. Vaping device. Electronic cigarette. Addiction.

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BENEFITS OF ABSTINENCE OF CHRONIC ALCOHOL EXPOSURE-INDUCED AGGRESSIVENESS IN CELLULAR MODELS OF HEPATOCELLULAR CARCINOMA

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Introduction: Hepatocellular carcinoma (hcc) is the most common type of primary liver cancer[1]. Alcohol-related liver disease is the most prevalent type of chronic liver disease worldwide, accounting for 30% of hcc cases and hcc-specific deaths[2]. Alcohol has been associated with an increased risk of several malignancies, this risk starting at doses as low as 10 g of pure ethanol/day. However, the pathophysiologic mechanisms underlying the effects of chronic alcohol exposure on the development of hcc and its aggressiveness are still unknown. Our study aimed to determine theses mechanisms (Constance Marié et al, in preparation).

Material and methods: We used a procedure of extensive exposure to alcohol (Chronic Alcohol Exposure, CAE) on two hcc cell lines: Huh-7 and SNU449. Hcc cells were exposed to alcohol during six months at different ethanol doses: 80, 160 and 270 mM. After this period, we pursued exposure or submitted cells to alcohol withdrawal. First, we characterized alcohol metabolism on the two cell lines. We investigated impact of alcohol and withdrawal on migration and invasive capabilities and on cell phenotype. Furthermore, we studied cancer stem cell markers, known as aggressiveness markers, in cells exposed to CAE and after withdrawal. Finally, we determined expression of these markers in a population of 68 hcc patients.

Results: Alcohol metabolism assays in SNU449 cells, in comparison to Huh-7 cells, revealed a reduction of alcetaldehyde synthesis by a decrease of alcohol deshydrogenase activity. Our results demonstrated also that CAE increases migration and invasive potentials. CAE also induced an aggressive phenotype acquisition in the early grade cell line Huh-7, close to that of the high grade cell line SNU449. Furthermore, CAE promoted expression of several cancerous stem cell markers: CD133, CD44, CD90 and CD24. Very interestingly, all modifications induced by CAE were partially or totally reversed by withdrawal. Our human study highlighted CD133 as specific of alcoholic hcc.

Conclusions: These results are in line with those of the epidemiological data suggesting the high risk of developing hcc in cirrhotic patients who maintain chronic and excessive alcohol consumption. Our study allows a better understanding of the mechanisms underlying decreased survival of patients with alcoholic hcc. They also demonstrated the importance of reinforcing interventions to target alcohol consumption and achieve alcohol abstinence in patients.

Keywords: hepatocellular carcinoma, cancer, alcohol, withdrawal, aggressiveness, cancerous stem cell

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Deschamps C., Uyttersprot F., Debris M**., Marié C.,** Fouquet G., Marcq I., Vilpoux C., Naassila M., Pierrefiche O. – **Delayed Memory deficit and impaired hippocampal synaptic plasticity after two binge drinking-life episodes during adolescence in rats is due to rapid and transient neuroinflammation**

Deep transcranial magnetic stimulation as a treatment for alcohol use disorder

Irene Perini

Background: Alcohol addiction is associated with a high disease burden, and treatment options are limited. We used deep repetitive transcranial magnetic stimulation (dTMS) to modulate activity in the insula and in the midcingulate cortex (MCC), two regions associated with the pathophysiology of alcohol addiction.

Methods: Two double-blind, randomized, sham-controlled, clinical trials were performed across two sites. At Linköping University, Sweden, fifty-six treatment-seeking patients were assigned to either sham or active TMS (10 Hz) targeting the insula bilaterally. At Ben-Gurion University, Israel, fifty-one patients were assigned to sham/active TMS targeting the MCC. For both studies, sham/active treatments were administered five times per week for three weeks. Craving and heavy drinking measures were collected at baseline, during treatment, and up to 12 weeks follow-up. Resting-state magnetic resonance imaging and task-based MRI data was collected before and after treatment.

Results: No significant effects of TMS stimulation targeting the insula were observed on craving and drinking measures at treatment and follow-up. However, a significant decrease in craving was observed at the end of TMS treatment targeting the MCC, which lasted up to 12 weeks post-treatment. In addition, self-reported heavy drinking was significantly decreased at follow-up. Finally, TMS of the MCC resulted in decreased resting state connectivity between MCC and the caudate.

Conclusion: In summary, while TMS targeting the insula did not show efficacy in alcohol addiction, TMS targeting the ACC shows some therapeutic potential.

S 21 Epidemiology, cancer and neurology in drinkers

Epidemiology of alcohol and opioids

Jürgen Rehm

The following topics will be covered in the overview:

1) Prevalence of alcohol and opioid use in 2019 by country, sex and age

2) Prevalence of alcohol and opioid use disorders in 2019 by country, sex and age

3) Drivers of prevalence within and between countries

4) The impact of COVID-19 on prevalence of both use and disorders

Implications for treatment, research and control policies will be sketched out.

Alcohol and Cancer: From Epidemiology to Molecular Mechanisms

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Alcohol consumption is the third leading risk factor for disease and mortality in Europe. It has also been associated with cancers of the oral cavity, pharynx, larynx, esophagus, liver, colon/rectum and female breast, wherein a dose-

response relationship appears to exist such that the risk of developing these cancers increases in proportion to the amount of alcohol consumed. Enigmatically, alcohol intake decreases the risk of thyroid cancer and lung cancer (with evidence most strongly supporting lower risk for light and moderate drinkers relative to non-drinkers) and does not appear to affect prostate cancer incidence. Based on these epidemiological data, it is clear that alcohol affects the various organs of the body differently. The precise mechanism by which ethanol induces cancer is not well understood. However, studies suggest that alcohol metabolites and/or enzymes associated with alcohol metabolism generate reactive oxygen species and alter cellular redox balance, cause DNA damage and epigenetic dysregulation, and modulate one-carbon metabolism. In addition, alcohol metabolites can cause a dysbiotic colorectal microbiome and enhance intestinal permeability, resulting in bacterial translocation, inflammation and immunosuppression. All of these effects can increase the risk of developing cancer. This presentation will address the topic of alcohol and cancer, and focus on epidemiological studies and the current state of knowledge regarding the mechanisms by which ethanol can induce cancer.

RARE MALIGNANCY AND ALCOHOL MISUSE

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Abstract:
POEMS syndrome is a rare clonal plasma cell disorder. POEMS is characterized by polyneuropathy, osteosclerotic myeloma. Organomegaly, endocrinopathy, monoclonal plasma cell disorder, and skin changes. Extremely elevated levels of serum vascular endothelial growth factor (VEGF) are characteristic of the syndrome. The link between the chronic alcohol consumption and this malignant conditions was not reported. Also no previous study has evaluated the influence of cytokine and chemokines or viruses in the severity and evolution of POEMS.
Objectives: 1-to demonstrate the utility of quantitative measurement of serum levels of vascular endothelial growth factor (VEGF) in the diagnosis of POEMS and the monitoring of therapeutic interventions and overproduction of pro-inflammatory cytokines; 2- to demonstrate the negative role of alcohol misuse in POEM's evolution.
Methods: We followed a patient with POEMS syndrome that misuse alcohol for 25 years. The median levels of VEGF 2500 pg/mL. Serum levels of cytokines and chemokines were compared between the patient with POEM, 80 patients with chronic hepatitis C virus (HCV) infection, 12 healthy controls, and 80 individuals with alcoholic liver disease (ALD). We quantified (ELISA pg/mL) the levels of VEGF, Interferon gamma (IFN-γ), Tumor Necrosis Factor alpha (TNF-α), Regulated-upon-Activation Normal-T-cell-Expressed and presumably-Secreted; (RANTES), and Nuclear Factor kappa-B (NFκB).
Results: In POEMS patient, VEGF levels were elevated versus control or other diseases, TNFα levels were higher versus control, but lower when compared with HCV or ALD patients. The patient underwent a detoxification period and a VEGF levels decreased with therapeutic intervention. When the patient begin drinking again, the disease relapse. The levels of proinflammatory cytokines and VEGF return to the initial values.
Conclusion: Chronic alcohol misuse can lead to rare malignancies such as POEMS syndrome. Extreme elevation of VEGF levels is diagnostic for POEMS syndrome, and should be followed to assess response to therapy. In addition, other co-morbidities should be considered individually to ensure personalized therapeutic intervention.

Wernicke-Korsakoff syndrome

Alain Dervaux, MD, PhD (Université Paris Saclay / EPS Barthélémy Durand Etampes)

Wernicke-Korsakoff syndrome is a complication of a thiamine (vitamin B1) deficiency, common in patients with alcohol use disorders. Wernicke's encephalopathy is underdiagnosed, up to 80% of cases, and undertreated. This syndrome is classically described as a clinical triad consisting of confusion, ocular dysfunction, in particular nystagmus and ophthalmoparesis, and ataxia. However, 16% of the patients with Wernicke's encephalopathy present with this complete triad.
Wernicke's encephalopathy is a clinical diagnosis but underdiagnosed because of the inconsistent clinical presentation and overlapping of symptoms with other neurological conditions. According to the European Federation of Neurological Societies (EFNS), the clinical diagnosis of Wernicke's encephalopathy in patients with alcohol use disorder requires two of the following four signs: 1) dietary deficiencies, 2) eye signs, 3) cerebellar dysfunction, and 4) either an altered mental state or mild memory impairment. MRI signal characteristics (symmetric alterations in the thalami, mammillary bodies, tectal plate, and periacqueductal area) are still not definitive for the diagnosis.
Wernicke encephalopathy is readily reversible if treated with adequate doses of parenteral thiamine, preferably within the first 48–72 h of the onset of symptoms. The overall safety of intravenous thiamine is very good. The risk of anaphylaxis is low. Substitution of parenteral thiamine in individuals with suspected Wernicke's encephalopathy is a well-established treatment regimen, but vary widely in available guidelines. Optimal dosing strategy and duration of the treatment remains unclear.
Untreated, Wernicke's encephalopathy can lead to coma or death, or progress to Korsakoff syndrome. Korsakoff syndrome is chronic and may be irreversible. characterized by cognitive and behavioral symptoms, including anterograde and retrograde memory impairments, executive dysfunction, confabulation, apathy, affective and social-cognitive impairments. To date, no effective pharmacological treatment for KS is available. The treatment of Korsakoff syndrome is based on cognitive rehabilitation, including memory compensation techniques, interventions based on errorless learning. Several studies using digital technologies to assist memory in patients with Korsakoff syndrome have shown promising results.

S 22 YIS 4 Liver disease

ALCOHOL USE DISORDER AND ALCOHOL RELATED LIVER DISEASE: TIME FOR SCREENING?

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Background and Aim: Alcoholic liver disease (ALD) causes approximately 3.3 million deaths worldwide every year and is one of the most common indications for liver transplant. Despite the great health burden, ALD is rarely detected in early stages. Non-invasive tests have been developed to determine the severity of liver disease in patients with alcohol use disorder (AUD). The **aim** of this study was to evaluate the severity of liver steatosis and liver fibrosis (LF) in a cohort of patients with AUD.
Methods: A prospective study was performed on 172 patients, without previously known liver disease, evaluated by AUDIT-C score, serum markers (TGO, TGP, platelets) and transient elastography (TE, FibroScan, Echosens) with Controlled Attenuation Parameter (CAP). AUD was defined by an AUDIT-C test score ≥4 for men and ≥3 for women. For LF evaluated by TE, the cut-off values proposed for ALD were used F2≥9kPa, F3≥12.1kPa, F4≥18.6kPa, while for liver steatosis by CAP the following cut-off values were used: S2>260db/m for moderate steatosis and S3>290db/m for severe steatosis

Four indirect scores were calculated and literature based cut-offs were used for the diagnosis of advanced liver fibrosis (≥F3): APRI≥1, FIB 4 ≥3.25, AST/ALT ratio ≥1 and Age-platelet index≥6.

Results:172 subjects with positive AUDIT-C test, 156/172 (90.70%) males, mean age 56.5±10.45 years were included. TE diagnosed advanced fibrosis (F3) in 13.9% (24/172) of the subjects and liver cirrhosis (F4) in 17.5% (30/172) of them. Moderate and severe steatosis was found in 18.6% (32/172), respectively 52.3% (90/172) patients. Statistically significant correlations were found between LS and AUDIT-C values (r=0.46, p<0.0001), APRI (r=0.33, ,p=0.001), FIB-4 r=0.31, p=0.0012) and the age-platelet index (r=0.25, p=0.008).

In univariate regression analysis, AUDIT-C (p=0.001), FIB-4 (p=0.01) and age-platelet index(p=0.03) were independently associated with the presence of advanced fibrosis advanced fibrosis (≥F3). In multivariate regression analysis only the model including AUDIT-C (p<0.001) and age-platelet index (p=0.04) was associated with advanced fibrosis (≥F3).

Based on AUROC comparison, age-platelet index (AUC-0.82) performed significantly better than AST/ALT (AUC-0.55) and APRI (AUC- 0.58) (p=0.0001 and p=0.0014, respectively), but no differences were found when compared to AUDIT-C (AUC-0.74) and FIB-4 (AUC- 0.77) (p=0.21 and p=0.35, respectively) for predicting advanced fibrosis.

Conclusions: in a cohort of patients with AUD**,** 70.9% presented moderate and severe liver steatosis and 17.5% were newly diagnosed with LC. These findings could be the basis for screening algorithms in the diagnosis of significant liver involvement in AUD.

Key words: *alcoholic liver disease, alcohol use disorder, screening, non-invasive techniques*

Role of Gut Hormones, Ghrelin and Liver-Expressed Antimicrobial Peptide-2 Dysregulation in Alcohol-Associated Liver Disease Pathogenesis

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Abstract:

Introduction: The pathophysiological mechanisms involved in development and progression of alcohol-associated liver disease (ALD) are complex and multifactorial, including dysregulated inter-organ (gut, pancreas and adipose tissue) crosstalk by peptides. In our previous work, we showed that ghrelin peptide is a stomach-derived "hunger" hormone that significantly increases with chronic alcohol exposure. Further, we demonstrated that an alcohol-induced increases in ghrelin reduces insulin and adiponectin secretion from pancreas and adipose tissue, respectively. These effects, mediated via the ghrelin receptors, cause increased mobilization of fatty acids from adipose to the liver and reduced adiponectin-mediated hepatic fatty acid oxidation, ultimately leading to the development of hepatic steatosis. In our recent study, we found that chronic alcohol administration decreases the circulating levels of liver-expressed antimicrobial peptide-2 (LEAP-2), a recently discovered endogenous ghrelin antagonist that is also expressed in the intestine. In this study, we investigated whether ghrelin decreases LEAP-2 levels similar to its effect on insulin and adiponectin. **Methods:** Adult male Wistar ghrelin receptor knockout (GHS-R KO) and wild type (WT) rats were pair-fed with Lieber-DeCarli liquid control or ethanol diets. After 6 weeks of feeding, rats were sacrificed, and blood and tissues were collected for analysis. In addition, in vitro studies were conducted where primary cultures of rat hepatocytes were treated with ghrelin. We also analyzed serum samples of ALD patients. **Results:** Ethanol-fed KO rats exhibited improved insulin sensitivity and serum insulin, normalized adiponectin and LEAP-2 levels and significantly reduced hepatic triglycerides compared to WT ethanol-fed rats. These results indicated that the ethanol-induced rise in serum ghrelin contributes to the decreased serum LEAP-2 observed. *In vitro* studies confirmed that indeed ghrelin impairs LEAP-2 secretion from hepatocytes which was also corroborated by reduced serum LEAP-2 levels patients with

ALD compared to controls. **Conclusions:** Alcohol-induced- increased serum ghrelin reduces LEAP-2 levels, a peptide capable of reducing GHS-R mediated signal transduction, thereby promoting its own efficacy to dysregulate multiple organs such as the pancreas and adipose function to ultimately lead to the development of fatty liver disease.

Elevated S-adenosyl-homocysteine-induced dipose dysfunction promotes alcohol-associated liver steatosis

Madan Kumar Arumugam

Distinct Mechanisms of Liver Cancers Between Alcoholic and Nonalcohlic Fatty Liver Diseases in Diabetic Murine Models

Liya Pi, Natacha Jn-Simon, and Chunbao Sun
(University of Florida Gainesville)

Background & Aims
Alcohol related liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD) share similar histologic features with liver pathologies ranging from steatosis, fibrosis, cirrhosis, to hepatocellular carcinoma (HCC). This study aims to determine whether the two types of diseases have common mechanisms in liver cancer development.
Methods
ALD or NAFLD was modeled in mice through feeding of ethanol-containing Lieber-DeCarli liquid (EtOH) or high fat diets (HFD) respectively. Prediabetes caused by streptozotocin administration was combined in the models to trigger HCC occurrence. RNA Sequencing was performed to compare regulations in cell cycle, stemness activation, and metabolic reprogramming between EtOH and HFD-associated murine tumors. The similarities of the two types of tumors to human HCC were examined in Gene Set Enrichment Analysis (GSEA).
Results
About 353 upregulated genes and 534 downregulated genes were identified in HFD-associated tumors while 749 upregulated genes and 436 downregulated genes were found in EtOH-associated tumors. Overexpression of similar gene clusters in cell cycle regulation was observed, indicating utilization of the same machinery for proliferation in the two types of tumors. However, distinct expression patterns of zonation-specific genes were observed. Seven stemness-related pericentral-specific genes in Wnt/ß-catenin pathways was upregulated in HFD-associated tumors but downregulated in EtOH-associated tumors, implicating "pericentralization" in HFD-associated tumors but "depericentralization" in EtOH-associated tumors. "Depericentralization" in EtOH-associated tumors was also supported according to downregulation of pericentral-specific genes in bile acid synthesis, phosphatidylcholine synthesis and drug metabolism. In particular, EtOH-associated tumors expressed low levels of Cyp2e1 and Aldh2, indicating impairment of ethanol metabolism. In contrast, HFD-associated tumors exhibited "deperiportalization" feature based on downregulation of periportal-specific genes involved in urea cycle, amino acid metabolism, and growth hormone signaling. GSEA indicated that HFD-associated tumors showed gene signatures similar to Hoshida subclass S3 in human HCC characterized by well-differentiated phenotypes, whereas EtOH-associated tumors showed gene signatures similar to Hoshida subclass S1 in human HCC that were proliferative, with activation of Tgf-ß2, C-myc and fibrotic pathway but low Wnt/ß-catenin activity.
Conclusions
Distinct stemness and metabolic reprogramming were found in HCC induced by HFD and EtOH in diabetic mouse livers.

Spleen stiffness/length to liver stiffness ratio significantly differs between ALD and HCV and predicts disease-specific complications

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Background: Both liver stiffness (LS) and spleen stiffness (SS) are widely used to non-invasively assess liver fibrosis and portal hypertension, respectively. However, the impact of disease etiology namely the localization of inflammation (portal versus lobular) on SS/LS remains unclear so far.
Methods: In a multicenter approach, LS and SS were prospectively assessed in 411 patients with ALD and HCV using Fibroscan (Echosens, Paris) including the response to treatment (alcohol withdrawal, HCV therapy). LS and spleen length (SL) were further analyzed in a retrospective cohort of 449 patients with long-term data on decompensation/death.
Results: Both, SS and SL were significantly higher in HCV as compared to ALD (42.0 vs 32.6 kPa, P<0.0001, 15.6 vs 11.9 cm, P<0.0001) despite a lower mean LS in HCV. Consequently, the SS/SL to LS ratio was significantly higher in HCV (3.8 vs 1.72 and 1.46 vs 0.86, P<0.0001) through all fibrosis stages. Notably, SL linearly increased with SS and the relation between SS and SL was identical in HCV and ALD. In contrast, livers were much larger in ALD at comparable LS. After treatment, LS significantly decreased in both diseases without significant changes of the SS/LS ratio. In the prognostic cohort, ALD patients had higher LS values (30.5 vs 21.3 kPa), predominantly presented for jaundice (65.2%) and liver failure was the major cause of death (P<0.01). In contrast, in HCV, spleens were larger (17.6 vs 12.1 cm) while variceal bleeding was the major cause of decompensation (73.2%) and death (P<0.001).
Conclusion: Both SS/LS and SL/LS ratio are significantly higher in patients with portal HCV compared to lobular ALD. Thus, combined LS and SS/SL measurements provide additional information about disease etiology and disease-specific complications.

S 23 From 3-R to 6-R: Improved ethics for the use of animals towards narrowing the translational gap in alcohol use disorder"

Refining animal models in the field of alcohol use disorder

Marcus Meinhardt (Central Institute of Mental Health, Germany)

Abstract:
Adequate animal welfare is linked to the quality of research data generated from laboratory animals, their validity as models of human disease and the reproducibility of animal studies. More than 60 years ago, Russell & Burch developed the 3R principle - Replacement, Reduction and Refinement – and these have become the guiding norm for animal welfare and the ethical use of animals in research. Scientists around the world have largely committed to the 3Rs and have recently been integrated into legislation in the EU directive 2010/63.
Identifying improvements of animal welfare and promoting these in the scientific community, is main goal of the 3R-Center Rhein-Neckar, Mannheim, Germany for animal experimentation in psychiatry. In detail, the 3R-Center Rhein-Neckar has targeted research activities to improve animal models in alcohol research. Especially concerning refinement, housing conditions in alcohol research have always been a matter of intense debate (e.g. single vs. group housing, enrichment). In this talk, we will address how to increase animal welfare in alcohol research, with a focus on refinements in rodent models of alcohol dependence. Ultimately, we will provide convincing arguments on higher animal welfare standards in alcohol research that are accompanied with an increase in data robustness.

THE EQIPD QUALITY SYSTEM: A NEW WAY TO BOOST INNOVATION

Björn Gerlach (PAASP GmbH, Heidelberg, Germany. On behalf of the EQIPD consortium)

Risk of failure is an inherent part of developing innovative therapies which can be reduced by adherence to evidence-based rigorous research practices. Supported through the European Union's Innovative Medicines Initiative, the Enhancing Quality in Preclinical Research (EQIPD) consortium has developed a novel preclinical research quality system that can be applied in both public and private sectors and is free for everyone to use.

The EQIPD Quality System was designed to boost innovation by ensuring the generation of robust and reliable preclinical data while being lean, effective and user-friendly. Consequently, the system should not become a burden that could negatively impact the freedom to explore scientific questions.

The system was developed over four years by 30 consortium partners from academia, industry, small to midsize contract companies and non-profit organizations. Additional 75 plus stakeholders provided feedback on aspects important for their environment. As a result, the EQIPD Quality System has been based on a set of 18 core requirements defining basic best practices applicable for any research lab. These core requirements are supplemented with 6 additional requirements which need to be applied when the research aims to make a "formal knowledge claim". The requirements can be addressed flexibly, according to user-specific needs and following a user-defined trajectory. The EQIPD Quality System proposes guidance on expectations for quality-related measures, defines criteria for adequate processes (i.e., performance standards) and provides examples of how such measures can be developed and implemented.

EQIPD has also developed tools (for optional use) to support users in implementing the system and to provide guidance for research units that have implemented the quality system and seek formal accreditation. In the first half of 2021, the first research units went through the assessment procedure and have received the first EQIPD certificates.

Building upon the feedback from users, a sustainable EQIPD Quality System will ultimately serve the global community of scientists conducting non-regulated preclinical research and will help them to generate reliable data that are fit for their intended use.

Keywords
EQIPD Quality System, rigorous research, core requirements, formal knowledge claim

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Reporting guidelines for non-clinical data

Kaitlyn Hair

The ARRIVE reporting guidelines were published in 2010 by the UK NC3Rs to encourage improvements in the reporting of animal research. Despite endorsement by journals, funders, and institutions, evidence of impact has been limited. In a randomised controlled trial in collaboration with PLOS ONE, we showed that mandating the completion of an ARRIVE checklist with manuscript submission had no impact on adherence to the guidelines in published papers. Recently, in line with our findings, the ARRIVE guidelines have been revised to enable easier adoption and evaluation by stakeholders. To accompany the revised guidelines, an elaboration document was produced with the aim of enhancing researchers' understanding of the key concepts. In this talk, I will discuss the rationale behind the ARRIVE reporting guidelines, their impact in practice, and how they can be used to improve rigour and reproducibility in animal research.

HOW TO COMBINE GOOD PLANNING WITH GOOD REPORTING TO IMPROVE THE VALIDITY, REPRODUCIBILITY AND TRANSLATABILITY OF PRECLINICAL RESEARCH

Adrian Smith (Norecopa, Ås, Norway, adrian.smith@norecopa.no)

Scientists have been criticised by the animal rights movement for decades, but more recently, scientists themselves have started to raise concerns about the reproducibility and translatability of preclinical in vivo research.

Efforts to improve the reproducibility of animal experiments have tended to focus on the more "mathematical" elements of experimental design such as the experimental unit,

randomisation, blinding and flaws in statistical analysis. These are undoubtedly the easiest metrics to collect, but they address only a small part of the potential for variability.

Experience over the last 30 years in running accredited laboratory animal facilities, supervising research and teaching Laboratory Animal Science has helped us identify many of the challenges in designing, conducting and reporting robust and valid animal studies. A group of Norwegian and British scientists has produced a set of guidelines, called PREPARE, to help in this process. PREPARE consists of a 2-page checklist, currently available in 25 languages, and a website with more information about each of the topics on the checklist. The website is updated continuously with links to new resources.

Scientists are encouraged to seek collaboration with the animal facility where they intend to work, from the earliest possible stage. This ensures that all aspects of the planned experiment, including the practicalities, timeframe, health and safety issues, distribution of labour and costs are discussed with all those who will be involved.

PREPARE is not intended to be yet another bureaucratic hurdle in the way of science. In fact, by alerting scientists to potential causes of variability at a very early stage, PREPARE should make it considerably easier to address questions posed to them later, for example during the publication process. A win-win-win situation for science, human safety and animal welfare.

We have made a 3-minute whiteboard film illustrating the principles in PREPARE, making comparisons with the aviation industry with their excellent record of safety and reliability (https://norecopa.no/prepare/film).

PREPARE is available at https://norecopa.no/PREPARE

Reference

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S 24 Legal aspects and management of acute disease

Legal aspects of alcoholic intake

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Introduction

Current data at european level show that alcohol dependence remains at alarming levels (5.4% of men aged 18-64 and 1.5% of women), and in Romania, it seems to decrease alcohol consumption from 17, 4 l (pure alcohol consumption) in 2000, to 12 l (pure alcohol consumption) in 2018. However, drivers and consumption in the general population are worrying due to road accidents or aggressions and also long-term consequences caused by alcohol.

Material and Methods

From the cases analyzed by GC-MS in 2018-20120, we selected the results of analyzes from biological samples collected from drivers involved in accidents or traffic controls and analytical results of blood alcohol levels determined in forensic autopsy cases.

Results

Out of a total of 2131 drivers, the police order to colect blood samples. The blood was collected with standard blood alcohol collection kits. In 263 of the cases, the people were not under the influence of alcohol at the time of the traffic accident and 366 cases people had blood alcohol levels of less than 0.80g ‰, but in 587 of the cases they had blood alcohol levels higher than 0.80g ‰. Romanian road code prohibits the consumption of alcohol in the case of drivers, so that those detected in traffic in an alcohol control test are verified by collecting blood samples. If the result determines a concentration higher than 0.80g ‰, it is considered a criminal offence.

In the forensic autopsy cases, 1618 biological samples were analyzed by GC MS, being negative 1050 cases, and 568 samples showed alcohol consumption. Known organic changes in chronic alcoholism have been observed: liver

steatosis, cirrhosis, dilated cardiomyopathy. We observed that against the background of alcohol consumption, murders were committed or murder followed by suicide. The forensic examinations were also for victims of domestic violence.

Conclusions

Alcohol consumption is a major problem due to its serious social effects (deaths due to complications, road accidents with human victims, domestic violence).

Of the group studied by drivers, only 21.31% did not consume alcohol, the rest drove under the influence of alcohol.

Keywords: alcohol consumption, drivers, criminal offence

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Romanian Penal Procedure Code

Romanian Road traffic Code

https://inml-mm.ro/?pg=pag/rapoarte

The pharmacological management of severe alcohol withdrawal syndrome

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According to WHO data, in 2016, the prevalence of alcohol use disorders and alcohol dependence in the Romanian population aged 15 and over, for the same year, were 2.8% and 1.3%, respectively. In addition, 11,844 people in Romania died due to alcohol-attributable causes, of these 6,366 died from liver cirrhosis, 802 were caused by road traffic injuries, and 4,676 due to cancer. Alcohol withdrawal syndrome (AWS) is a frequent complication of those who met the criteria for alcohol dependence. Its intensity may vary from symptoms such as insomnia, tremors, sweating, tachycardia up to more severe complications such as delirium or seizures, implying 5-15% mortality.

Currently, there are two main directions in the pharmacological management of AWS. First, symptom-triggered therapy is when treatment is provided if the symptoms are severe but not if the symptoms are mild. In this latter case, the simple continuing monitoring without medication is sufficient. Therefore, the unnecessary use of benzodiazepines or phenobarbital is avoided. Second, in those being delirious or having a prior history of Delirium Tremens or seizures, a fixed-dose regimen of some type needs to be administered. On the other hand, the pharmacological treatment of AWS should cover three symptomatic domains: neuropsychiatric symptoms, autonomic symptoms, and motor disturbances.

Finally, severe AWS should be considered as a psychiatric emergency that emerges progressively. Therefore, the mainstay of alcohol withdrawal management is early adequate treatment.

Microbiota and alcoholic liver

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The human microflora - "microbiota" – consists of multiple species of germs (bacteria, fungi or viruses) that colonize the skin, genitourinary system, respiratory system and digestive tract. The largest microbial density is in the bowel, representing the intestinal microbiota. The intestinal microbiom includes about 1000 different species of germs, and has several functions:

- **External barrier („barrier effect"),** preventing pathogen germs from colonizing the intestinal mucosa. Secondary dysbiosis due to antibiotics allows the colonization of the mucosa by *Clostridium difficile*.
- **Metabolic and energetic („metabolic organ") function**, producing energy from the undigested residues from the small bowel („recoveres residues"). Under the action of bacterial flora result

short-chain fatty acids, mainly butyric acid, with beneficial role on colonocite.
- **Maturation and education of intestinal immune system** by continuous stimulation, direct and indirect, and by producing a mild chronic physiological inflamation ("low –grade physiological inflammation"), that maintains a perfect symbiosis with the microbiota („innate and adaptive immune responses"). Disturbance of the interaction between the host and the microbiota may result in an inappropriate or exaggerated inflammatory response, with the increase mucosal permeability.

Disbiotic changes in intestinal microbiota occur in most liver disease, suggesting both their contribution to chronic hepatopathy pathogenesis, but also the adverse effect of the disease on the microbioma. In alcohol related liver disease (ALD) there is an increased dysbiosis, an increased permeability of the intestinal wall and an increase small bowel bacterial overgrowth.

Intestinal disbiosis in patients with alcoholic hepatopathy leads to increased intestinal permeability and bacterial translocation and endotoxinemia, but also generates immune system disorders, increasing liver inflammation and inducing modifications of microbial metabolism, especially in transformation of bile acids.

Patients with ethanolic hepatic cirrhosis had a high abundance of Enterobacteriaceae and Halomonadaceae, a low abundance of Lanchospiraceae, Ruminococcaceae and Clostridialies XIV, increased endotoxins' levels and a lower ratio of abundance of microbial types "Good vs. Bad "(called cirrhosis disbiosis ratio [CDR]).

The intestinal microbiota is closely related to the initiation and progression of hepatic lesions in patients with abusive alcohol consumption. Specific changes to intestinal microbiota can increase the progression of the hepatic lesions by changes in microbial function, especially the metabolism of bile acids. But there are still many questions regarding the relation between microbiota and chronic liver diseases, including ALD.

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S 25 Modifiers of alcoholic liver disease

Alcohol-related liver disease, hemolysis and iron signaling

Shijin Wang, MD and Sebastian Mueller, MD, PhD

Background and aims: The liver is the main target organ of heavy alcohol consumption eventually causing alcohol-related liver disease (ALD), but our recent study indicates that red blood cells (RBC) are also an important target in alcoholic injury, and the dysfunctional liver cells can aggravate. **Methods:** Based on a large prospectively enrolled patient cohort of heavy drinkers (Heidelberg cohort), we identify a subgroup of 25% with high ferritin and low hemoglobin levels that show the worst outcome. Mice models of mild and severe hemolysis were built by once or double phenyl hydrazine (PHZ) injection. In vitro, we used endothelial cells and hepatocytes co-culture to observe heme effects on hepcidin regulation.

Results: In subgroup of patients with low hemoglobin and high ferritin, the large erythrocytes (high MCV), elevated unconjugated bilirubin and LDH before or after the detoxification are highly suggestive of enhanced hemolysis. This is further confirmed by the high expression of CD163, a heme scavenger. Surprisingly, although challenge with the hemolytic agent phenyl hydrazine (PHZ)

demonstrated elevated RBC fragility in ALD patients, levels of folic acid and B12 were comparable to controls. Moreover, despite iron overload in these patients, serum levels of the iron master switch hepcidin were suppressed ultimately causing further iron uptake. In mouse models, mild hemolysis induced hepcidin while severe hemolysis causes a paradox downregulation. Finally, in vitro heme strongly upregulates endothelial cell-derived BMP6, the most important signaling molecule upstream of hepcidin. In contrast, iron release from severe heme degradation causes a direct blockage of hepatocellular BMP/SMAD signaling pathway.
Conclusion: Our translational findings suggest a novel role of heme turnover in hemolytic damage of patients with ALD and systemic iron overload due to altered hepatocellular hepcidin signaling, which then forms a vicious circle between blood and liver.

Intestinal bacteria and bacterial endotoxin in the development and therapy of alcohol-related liver disease

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Alcohol intake is still among the leading causes of chronic liver diseases world-wide. Despite intense research efforts made, the understanding of the molecular mechanisms underlying the development of alcohol-related liver diseases are still limited and universally accepted therapies and prevention measures mainly focus on abstinence often afflicted with high relapse rates. Changes in intestinal microbiome and barrier function are discussed to be critical in the development of alcohol-related liver disease (ALD), too. Indeed, results obtained from studies in model organisms but also humans suggest that acute and chronic intake of higher doses of alcohol can lead to an increased translocation of bacterial toxins and herein especially bacterial endotoxin being also associated with intestinal barrier dysfunction e.g., a loss of tight junction proteins and changes of intestinal microbiota. Furthermore, experimental studies suggest that targeting intestinal microbiota and barrier function in settings of acute and chronic alcohol intake be it through a treatment with probiotics, secondary plant compounds or amino acids may improve alcohol-related liver diseases. Also, it has recently been shown that in humans even a short period of total abstinence can improve not only markers of alcohol-related liver disease but also those of intestinal barrier function. Here, an overview summarizing recent findings and highlighting emerging trends in the interaction of gut and liver with a specific focus on intestinal barrier function and bacterial endotoxin will be given.

Role Of Environmental Toxins And Nanoplastics In The Pathogenesis Of (N)AFLD

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Background: Alcoholic liver disease (ALD) and non alcoholic liver disease (NAFLD), the most common liver diseases worldwide, show identical histology and also share common genetic and non-genetic progression factors. Especially obesity and diabetes are important confounders of both diseases suggesting that food-related toxin exposures could be involved. This study was aimed at gaining first data on the in vitro toxicity of environmental pesticides and nanoplastics in cultured hepatoma cells.
Methods: A novel high content *in vitro / in silico* toxicology platform was deployed to screen toxic ramifications in *HepaRG* cells that were previously exposed to several pesticides commonly taken up through ingestion. In addition, the effect of environmental nanoplastics (Polystyrene particles of 25nm, 100nm, 3µm) were studied, which are able to cross membranes of epithelial tissues of human lung, stomach and guts into the lymph and circulatory system[1,2]. For these particles, a simulation of chemical and physical weathering was applied in order to compare adverse effects of primary vs. secondary particles on hepatic cells and to

hence resemble more realistic exposures than previously studied in literature[1,3].

Results: Using this *in vitro/in silico* approach, we were able to observe significant changes after exposure to selected pesticides. In contrast, *HepaRG* cells appeared to be resistant against primary nanoplastics of tested sizes regarding viability and sublethal endpoints. However, it could be shown that when looking at viability parameters and oxidative stress assays, negative effects were observed in *HepaRG* cells when nanoparticles had undergone simulated weathering prior to cell exposure: In the case of exposure to weathered 3µm PS particles, viability of cultured cells sank by up to 86.5% (±12.2) whilst oxidative stress increased by up to 179.7% (±23.7) when compared to cells treated with unaltered particles. Moreover and in confirmation of previous work, our data suggest that nanoplastics of up to 3µm seem to require endocytosis[4].

Conclusion: We here show that cultured heaptoma cells tolerate even higher concentrations of primary Polystyrene nanoparticles but UV light modification of nanoplastics (weathering) induce a cellular response with regard to stress parameters. Further studies are needed to elucidate underlying molecular mechanisms to better understand the role of nanoplastics on the progression of (N)ALD.

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Influence of hepatic preinjury on development of ACLF in alcohol-related and other chronic liver disease

Steven Dooley, Tao Lin, Rilu Feng, Sai Wang, Honglei Weng et al.

One important response of the liver to damage is regeneration. After acute liver injury, hepatocytes are the principle cells responding to regenerative stimuli sent out from the environment, importantly from the non parenchymal cell populations, including Hepatic Stellate Cells, Kupffer cells and Liver Sinusoidal Endothelial Cells. In such setting, hepatocytes proliferate rapidly to restore the liver mass and simultaneously providing all functions necessary to maintain liver function and body homeostasis. Data from lineage tracing show that this scenario is hold throughout any liver diseases scenario, where sufficient amounts of healthy hepatocytes are still present. However, in circumstances of advanced cirrhotic disease, as is the case in severe Alcoholic Hepatitis, characterized by, among others massive loss of hepatic mass, the remaining hepatocytes lose proliferative capacity, and/or the few proliferating hepatocytes are not able to restore hepatic mass and maintain liver function. In such emergency situations, liver progenitor cells (LPC) in the Canals of Hering and the smallest branches of the biliary tree, proliferate rapidly and are able to transdifferentiate into hepatocytes in order to restore the parenchymal compartment. In an approach to identify parameters and mechanisms involved in (i) the deficiency of remaining hepatocytes to maintain essential liver functions and driving acute on chronic liver failure establishment, and (ii) on LPC activation and differentiation towards replacement of lost hepatocytes as well as take over of essential liver functions, we have studied liver and blood samples of patients with late stage liver disease, including Alcoholic Hepatitis, and have used liver LPC lines and primary LPC for functional studies. We found that even in irreversible ACLF, LPCs still are able to differentiate to hepatocytes. However, these LPC-derived hepatocytes do not provide sufficient hepatocyte function required for systemic homeostasis. We demonstrated a crucial role of lineage-determining transcription factor HNF4α in expressing key hepatocyte genes, e.g. albumin and coagulation factors in the arising LPC of these patients. Mechanistically, HNF4α expression in LPCs requires two variant transcription factor complexes, namely TRIM33-SMAD2/3 and FOXH1-SMAD2/3/4,

which are driven by extracellular Activin signaling. Thereby, Activin does not alter the epigenetic phenotype, e.g., H3K4me3, H3K27me3 and H3K27ac, which we originally hypothesized in expectation of cellular fate changes. Instead, we found that LPC are reprogrammed to take over several essential hepatocyte functions, including the production of coagulation factors. In ACLF patients, presence of an intact Activin-HNF4α axis in LPCs can only be found in surviving patients. Further, we found that Activin levels alone can not be used as indicator for good or bad prognosis, instead the ratio of Activin and Follistatin has more relevance, since high levels of Follistatin can interfere with Activin signaling. Follistatin levels are controlled by the glucagon-insulin balance. Our results indicate a key role for the generation of an Activin signal-driven, HNF4α dependent fate modulation of newly generated LPC in severely predamaged livers to initiate liver regeneration and avoidance of liver failure and death.

S 26 Alcohol and Older Adults: Insights from Behavioral and Brain Studies

The average age of the world's population is continuing to increase resulting in the need to better understand health factors that may impact the aged population. Older individuals consume alcohol often in a dangerous, binge pattern. However, the impact of alcohol consumption on behavior and brain function in the aged population is understudied. The present symposium will highlight recent data revealing the extent of alcohol consumption in the aged population, the impact of alcohol exposure on human and animal behavior and begin to identify possible central nervous system mechanisms underlying the behavioral effects. Given the increase in the number of aged individuals coupled with alcohol use in this demographic, research focused on understanding the effects and mechanisms of alcohol in aged people are critical.

Chairs:
Douglas B Matthews, University of Wisconsin – Eau Cliare
Sara Jo Nixon, University of Florida

Epidemiology of Harmful Drinking Among Older Adults: Life Course Origins and Public Health Implications

Katherine M. Keyes, PhD (Columbia University, kmk2104@cumc.columbia.edu)

Alcohol consumption is increasing in the United States, as is alcohol-attributable mortality. The majority of alcohol-related chronic disease morbidity and mortality is concentrated among those in older age. Historically, men have had higher rates of alcohol consumption than women, though evidence for birth cohort effects on gender differences in alcohol consumption and alcohol-related harm suggest that gender differences may be diminishing. Yet available evidence indicates that the changes over time in alcohol consumption are widely variable by developmental life stage. Among adolescents and young adults, for example, both males and females are rapidly decreasing alcohol consumption, binge and high intensity drinking, and alcohol-related outcomes, with gender rates converging because males are decreasing consumption faster than females. This pattern does not hold among adults, however, and especially does not hold in older adults. Our research program has demonstrated that in middle-adulthood, consumption, binge drinking, and alcohol-related harms are increasing, driven largely by increases among women in their 30s and 40s. The trend of increases in consumption that are faster for women than men appears to continue into older adult years (60 and older) across several studies. Women in the US are increasing more rapidly than men in alcohol-related acute and chronic disease injury related to alcohol consumption, including liver disease. These rapid increases in alcohol consumption among older adult women in the US are concentrated among those with the highest levels of education and occupational prestige, and those

with the highest levels of education. Furthermore, the patterns of drinking are heterogeneous across the world. In our research among older adults across more than 20 countries, alcohol consumption patterns, and gender differences in drinking, vary considerably. These variations are associated with alcohol policy and drinking norms, but are also associated with broader trends in chronic disease and mortality, social safety nets and supports, and loneliness and depression across the world. Taking both a life course, birth cohort, and global alcohol and social policy perspective is necessary to understand, track, and intervene on harmful alcohol use patterns among older adults in order to address public health needs. Fundamentally, addressing the increase in alcohol consumption among adults using epidemiological surveillance data is an important and growing public health need.

Age-related Vulnerability at Socially-Relevant Doses

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A large literature addresses the effects of acute alcohol consumption in younger adults and another the effects of moderate drinking lifestyles in middle-to late adulthood. However, little work addresses the fact that moderate drinking lifestyles are punctuated by acute bouts of drinking. Thus, as the proportion of older adults increases and the number of older adults continuing to consume alcohol increases, it is incumbent on us to ascertain the neurobehavioral processes impacted by socially-relevant alcohol doses in older adults. Although limited, current data offer provoking insight. In this presentation, we discuss current data comparing older and younger healthy drinkers under active and placebo alcohol conditions across well-characterized neuropsychological tests, neurophysiological measures, and driving simulation. Results reveal a complex interaction with older and younger drinkers producing divergent results, characterized by differential benefit and compromise conditioned on task, dose and outcome measured. Together these data support the prediction of an age by alcohol interaction, consistent with differential impairment in top-down cognitive processes and which carries significant implications for neurobehavioral flexibility and adaptation.

Neuroinflammation al the intersection between alcohol and aging

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Binge-like alcohol consumption remains most prevalent among adolescents and continues to be a common area of study in both clinical and preclinical (rodent) model systems. However, rates of heavy drinking among middle-aged and elderly have increased substantially over the past decade as the global population ages, calling for the development of tractable animal models to better understand the influence of chronic alcohol consumption on overall brain health (Deak & Savage, 2019). To this end, our lab has utilized several approaches to better understand the influence of alcohol exposure across the lifespan. Studies first examined ethanol sensitivity (via social interaction test) and ethanol clearance rates among Fischer-344 (F344) rats at the ages of 3, 12 or 18 months of age, corresponding to young adult, middle aged, and late aging. Across a range of ethanol doses (0.5-3.5 g/kg ip), studies reported enhanced behavioral sensitivity to ethanol evidenced by a leftward shift in the dose response function among aged rats tested in both social interaction (low dose range) as well as Loss of Righting Reflex (high dose range) (Perkins, 2018). Few, if any, of these changes correlated to ethanol elimination rates, since differences in ethanol clearance did not begin to emerge until doses exceeded 1.5 g/kg (ip). Some minor sex differences were observed between aged males and females. Despite greater sensitivity to ethanol, aged rats consumed equivalent quantities of alcohol when given a 2-bottle choice procedure. In a parallel set of studies, aged rats displayed heightened expression of several inflammation-related signaling molecules (IL-6, MCP1) under basal conditions, but ethanol-induced changes in cytokines were largely absent among aged rats (compared to young adults; Gano et al., 2017). Ongoing studies are utilizing a single-bottle access model (10% ethanol, 2 days on, 2 days off) across the lifespan to better understand the influence of

chronic ethanol intake on (i) neuroinflammatory markers; (ii) cognitive dysfunction; and (iii) cellular markers of Alzheimer's Disease (AD; tau, b-amyloid plaques) among both wild type and TgF344-AD rats. Overall, these studies provide a foundation for pursuing mechanistic studies to better understand the relationship between brain aging, neuroinflammation, and alcohol across the lifespan.

Keywords: aging, alcohol, neuroinflammation, cytokine, microglia

Increased sensitivity to ethanol in aged animals: potential neurobiological mechanisms

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The number of older individuals in most, if not all, countries is increasing at a dramatic rate and this increase has the potential to strain the health care system. Understanding behaviors that may impact the health of the older population is therefore critical. Older adults consume alcohol, often in a binge pattern. Therefore, investigating if alcohol produces greater effects in the aged population compared to younger populations is of paramount importance. Preclinical animal models are useful to investigate behavioral effects following ethanol exposure and potentially identify central nervous system mechanisms underlying these differential responses. Recently our laboratory has been investigating if acute and chronic ethanol exposure produces greater effects in aged rodents compared to younger rodents. Converging lines of evidence from multiple different behavioral and cognitive measures reveal that aged rats are significantly more sensitive to the acute effects of ethanol compared to younger animals. The differential behavioral effects produced by acute ethanol in aged animals are not due to differential blood ethanol levels suggesting a central nervous system mechanism(s). In addition, short-term, chronic ethanol exposure impairs behavioral flexibility in aged rats, and the impairment in behavioral flexibility is selective to aged, but not adult, animals. Recent neuroproteomic data from hippocampus of treated animals reveals potential protein changes that may underlie the differential sensitivity to ethanol and reduced behavioral flexibility. Ongoing work in the laboratory is pursuing two directions: 1) Investigating protein pathway analysis that may explain the reduction in behavioral flexibility in aged rats, and 2) Biochemical mechanisms that may explain the increased sensitivity to acute ethanol in aged rats.

S 27 Alcoholism and the heart liver axis

Ethanol and heart failure

Adina Ionac

French Paradox – truth or challenge

Gaita Dan

Elastography for screening alcoholic liver disease (ALD)

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Abstract

Worldwide, alcohol abuse is associated with 3.3 million deaths every year and alcohol-related liver disease (ALD) is the most frequent cause of severe liver disease in Europe. WHO affirm that European Region has the highest adult per capita alcohol consumption and in the EU, 41% of liver deaths are attributed to alcohol.

From the morphological point of view, steatosis is present in almost all heavy drinkers and it is estimated that only 10–20% will eventually develop cirrhosis. Looking to the presence of advanced fibrosis or cirrhosis in compensated patients, this is the main predictor of long-term survival. From this point of view, the assessment of liver fibrosis for the prognosis is mandatory.

Looking to the OMS criteria ("screening test should offer high validity, reliability and acceptance in the screening population, in addition to a positive balance between yield and costs"), than ultrasound based elastography is

with high reliability, with high accuracy (between 80 and 95%, increasing with severity of fibrosis), well accepted by patients, not very expensive (and repetitive).
Transient Elastography (TE) is used for some years for this purpose, but point SWE (pSWE) and 2D-SWE (2D-SWE), , being included in an ultrasound machine, offer more capacities for liver evaluation.
Because up to 90% of patients with heavy alcohol intake have steatosis, and because steatosis has been identified as an independent predictive factor of fibrosis progression in heavy drinkers, assessment of steatosis can be performed with ultrasound (US), that is accepted as an initial screen for fatty liver, because it is non-invasive, being inexpensive and widely available. US has a sensitivity of 60–94% and a specificity of 88–95% in detecting steatosis. But, US sensitivity significantly varies according to degrees of fatty load, with 80% sensitivity at fat accumulation above 30%, and only 55% when the fat content reaches only 10–20%.
Fibroscan (Echosense, Paris), with M and XL probe for fibrosis and CAP (Controlled Attenuation Parameter) for steatosis, is used in practice in ALD patients and in a European multicenter prospective study, including 562 patients with ALD who underwent CAP, regular US and liver biopsy, CAP gave this results: mild steatosis – AUROC 0.77, moderate steatosis – AUROC 0.78 and severe steatosis - AUROC 0.82, and a CAP value > 290 dB/m ruled in any steatosis with 88% specificity. But more recent, quantification of steatosis (and fibrosis) can be performed using advances US systems (UGAP from GE, ATT from Hitachi or ATI from Canon), with high accuracy.
But, in ALD patients, assessment of fibrosis is the most important element. For this, TE was used for many studies. In a systematic review and meta-analysis of Pavlov et al. (Cochrane review), who aimed to assess the diagnostic utility of TE in ALD patients (with five retrospective and nine prospective cohort studies, with 834 participants), the authors could not identify the optimal cut-off values for the fibrosis stages. But, in a meta-analysis performed by Nguyen-Khac E et al, the cut-off for F4 range from 11,5 till 22,6 kPa, with an AUROC of 0,86 - 0,94. Than for TE the problems that must be solved are the optimal cut-off values for significant fibrosis (that range from 7.8 to 9.6 kPa) and severe fibrosis (that range from 8.0 to 17.0 kPa), in some studies. Some confounding factors (like AST and bilirubin) and alcohol withdrawal sometime before the evaluation, must be followed.
One important fact, shown in a study of Muller s et al. is that in the absence of elevated aminotransferases, cut-off values in TE were almost identical between HCV and ALD patients (in a cohort of 677 patients with ALD and 1391 with HCV infection). In this moment exist a proposed algorithm for alcoholic liver disease, where TE <6 kPa is used for exclusion of liver fibrosis and >12,5 kPa for liver cirrhosis diagnosis (Moreno C, Mueller S, Szabo G).
In some small studies, 2D-SWE and pSWE were used with quite good results for liver stiffness evaluation in ALD patients (2D-SWE.SSI gave comparative results with TE).
But some results from the TE screening! In a French elastographic screening study in more than 1,000 apparently healthy people >45 years, 7.5% had a pathologically increased liver stiffness > 8 kPa and in 36% of cases t,his was eventually linked to ALD.
But which are the recommendations of EFSUMB and WFUMB (European and World Federation of Ultrasound)? SWE (Shear Waves Elastography) can be used for liver stiffness assessment in ALD patients, to rule out advanced disease.
In conclusion, ultrasound based elastography methods can be used for liver assessment in ALD patients, but more studies must be performed to establish exact the place of this methods.

Alcoholic cardiomyopathy: Pathogenic aspects

Joaquin Fernandez Sola

Revisiting the role of HVPG and transjugular liver biopsy in alcoholic hepatitis

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Somehow, the role of liver biopsy in the diagnosis of alcoholic hepatitis was recently challenged by an extensive randomized control trial that failed to demonstrate a favorable effect of any treatment intervention in alcoholic hepatitis. At the same time, there is evidence that liver histology has prognostic relevance in alcoholic hepatitis. Usually, liver biopsy is performed by the transjugular route, which offers the opportunity to measure hepatic venous pressure gradient (HVPG), another predictive tool in the evaluation of these patients. However, the evidence about the role of HVPG is still scarce. We will review the role of histology and hemodynamic assessment in the management of alcoholic hepatitis.

POSTER PRESENTATIONS

REDUCED ALCOHOL INTAKE FOLOWING A COMBINATION THERAPY OF AMYLIN AND GLUCAGON-LIKE PEPTIDE-1 RECEPTOR AGONISTS IN MALE AND FEMALE RATS

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Objectives

New treatment options for alcohol use disorder (AUD) are warranted, as the effect of existing pharmacotherapies is suboptimal. Recent advances have pinpointed anorexigenic gut-brain peptides, such as glucagon-like peptide-1 (GLP-1) and amylin, as novel candidates. Indeed, amylin receptor (AMYR) agonists or GLP-1 receptor (GLP-1R) agonists independently reduce alcohol intake in male rats. Albeit a combination therapy of AMYR and GLP-1R agonists additively reduces food intake and body weight in male rats, the effects of a combination therapy of AMYR and GLP-1R agonists on alcohol intake in male and female rats are to date unknown.

Materials and methods

The present study was designed to evaluate the ability on the AMYR agonist salmon calcitonin (sCT), and the GLP-1R agonist dulaglutide, either independently or in combination to reduce the intake of alcohol, water and food, as well as body weight in both sexes. Male and female rats were exposed to the intermittent access alcohol two-bottle choice-drinking paradigm for 12 weeks. The rats were injected repeatedly with dulaglutide, sCT or a combination thereof and the aforementioned parameters were measured.

Results

A profound decrease in alcohol intake was noted in both female and male rats treated with sCT, dulaglutide or in combination. Moreover, both the monotherapies and sCT and dulaglutide together increased water intake in both sexes. Lastly, in both males and females, sCT and dulaglutide reduced food intake and body weight gain, and the combination therapy additively suppressed these parameters.

Conclusions

Collectively, our data indicate that a combination of sCT and dulaglutide should be evaluated as a potential treatment option for obese AUD patients.

Keywords

Alcohol use disorder, amylin, GLP-1, gut-brain peptides

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BENEFITS OF ABSTINENCE OF CHRONIC ALCOHOL EXPOSURE-INDUCED AGGRESSIVENESS IN CELLULAR MODELS OF HEPATOCELLULAR CARCINOMA

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Introduction: Hepatocellular carcinoma (hcc) is the most common type of primary liver cancer[1]. Alcohol-related liver disease is the most prevalent type of chronic liver disease worldwide, accounting for 30% of hcc cases and hcc-specific deaths[2]. Alcohol has been associated with an increased risk of several malignancies, this risk starting at doses as low as 10 g of pure ethanol/day. However, the pathophysiologic mechanisms underlying the effects of chronic alcohol exposure on the development of hcc and its aggressiveness are still unknown. Our study aimed to determine theses mechanisms (Constance Marié et al, in preparation).

Material and methods: We used a procedure of extensive exposure to alcohol (Chronic Alcohol Exposure, CAE) on two hcc cell lines: Huh-7 and SNU449. Hcc cells were exposed to alcohol during six months at different ethanol doses: 80, 160 and 270 mM. After this period, we pursued exposure or submitted cells to alcohol withdrawal. First, we characterized alcohol metabolism on the two cell lines. We investigated impact of alcohol and withdrawal on migration and invasive capabilities and on cell phenotype. Furthermore, we studied cancer stem cell markers, known as aggressiveness markers, in cells exposed to CAE and after withdrawal. Finally, we determined expression of these markers in a population of 68 hcc patients.

Results: Alcohol metabolism assays in SNU449 cells, in comparison to Huh-7 cells, revealed a reduction of alcetaldehyde synthesis by a decrease of alcohol deshydrogenase activity. Our results demonstrated also that CAE increases migration and invasive potentials. CAE also induced an aggressive phenotype acquisition in the early grade cell line Huh-7, close to that of the high grade cell line SNU449. Furthermore, CAE promoted expression of several cancerous stem cell markers: CD133, CD44, CD90 and CD24. Very interestingly, all modifications induced by CAE were partially or totally reversed by withdrawal. Our human study highlighted CD133 as specific of alcoholic hcc.

Conclusions: These results are in line with those of the epidemiological data suggesting the high risk of developing hcc in cirrhotic patients who maintain chronic and excessive alcohol consumption. Our study allows a better understanding of the mechanisms underlying decreased survival of patients with alcoholic hcc. They also demonstrated the importance of reinforcing interventions to target alcohol consumption and achieve alcohol abstinence in patients.

Keywords: hepatocellular carcinoma, cancer, alcohol, withdrawal, aggressiveness, cancerous stem cell

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Publications

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Fouquet G., **Marié C.**, Badaoui M., Demey B., Radoslavova S., Telliez M-S., Dhennin-Duthille I., Bayry J., Sevestre H., Ouadid-Ahidouch H., Marcq I., Bouhlal H. - **Mammary SLAMF3 Regulates**

Store-Operated Ca2+ Entry and Migration Through STIM1 in Breast Cancer Cells and Cell Lines - Journal of Cancer Science and Clinical Therapeutics - 4 (3): 214-231 – 2020

In submission:

Marié C., Fouquet G., Collet L., Duroyon V., Vilpoux C., Ouled-Haddou H., Nguyen-Khac E., Naassila M., Bouhlal H., Marcq I. - **SLAMF3 enhances Sorafenib response in HCC cells through induction of Mesenchymal to Epithelial Transition (MET)**

In preparation:

Marié C., Fouquet G., Nguyen-Khac E., Naassila M., Bouhlal H., Marcq I. - **Benefits of abstinence on chronic alcohol exposure-induced aggressiveness in cellular model of hepatocellular carcinoma This abstract is taken from this article.**

Deschamps C., Uyttersprot F., Debris M**., Marié C.,** Fouquet G., Marcq I., Vilpoux C., Naassila M., Pierrefiche O. – **Delayed Memory deficit and impaired hippocampal synaptic plasticity after two binge drinking-life episodes during adolescence in rats is due to rapid and transient neuroinflammation**

PHYSICAL EXERTION AT WORK AND ADDICTIVE BEHAVIORS: TOBACCO, CANNABIS, ALCOHOL, SUGAR AND FAT CONSUMPTION: LONGITUDINAL ANALYSES IN THE CONSTANCES COHORT

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Objectives

This study examined the prospective association of physical exertion at work with risk of tobacco, cannabis, alcohol use and sugar and fat consumption.

Materials and methods

Volunteers of the French population-based CONSTANCES cohort currently employed were included from 2012 to 2017 for tobacco and cannabis outcomes (n=100,612), and from 2012 to 2016 for alcohol and sugar and fat outcomes (n=75,414). High level of physical exertion was defined as a score ≥12 at the Rating Perceived Exertion Borg scale. Substance use was self-reported and patterns of sugar and fat intakes were obtained from principal component analysis and used in quartiles. Generalized linear models computed odds of substance use and sugar and fat consumption at follow-up according to baseline physical exertion at work, while adjusting for sociodemographic factors, depressive symptoms and baseline level of consumption.

Results

High physical exertion was associated with tobacco use, i.e.: increased odd of relapse in former smokers (OR=1.13, 95% confidence interval (CI):1.02-1.24), and increased number of cigarettes per day in current smokers (OR=1.54, 95%CI:1.33-1.78) with dose-dependent relationships (P for trend

Conclusions

The associations between physical exertion at work and subsequent tobacco and cannabis use and sugar and fat consumption should be taken into account for information and prevention strategies.

Keywords

physical exertion; alcohol; cannabis; tobacco; sugar and fat; employees; epidemiology

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RO 61-8048, A SELECTIVE INHIBITOR OF KYNURENINE MONOOXIGENASE, REDUCES ETHANOL CONSUPTION IN DEPENDENT MICE

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Objectives

Alcohol is the most consumed drug in the world. The kynurenine (KYN) pathway, the main route of tryptophan metabolism, has been recently proposed as a new target for modulating drug abuse [1]. We have previously demonstrated that inhibition of the enzyme kynurenine 3-monooxygenase (KMO) using Ro 61-8048 is able to reduce EtOH consumption in a binge drinking model [2]. However, to date, there is no evidence on the effect of kynurenine pathway modulation in animals addicted to alcohol.

Materials and methods

Adult male and female mice were subjected to the Chronic Intermittent Ethanol (CIE) paradigm, an EtOH dependence and relapse drinking model [3]. On the last day of CIE, mice were treated with Ro 61-8048, Ro 61-8048 + PNU120596 (a positive allosteric modulator of α7nAChR), Ro 61-8048 + L-leucine (competes with KYN for LAT1, which transports KYN from the periphery into the brain) or Ro 61-8048 + probenecid (blocks the OAT, the transporter that extracts KYN from the brain). EtOH and water consumption and preference for EtOH were measured and KYN concentrations in both plasma and limbic forebrain were determined by HPLC.

Results

Ro 61-8048 decreases consumption of and preference for EtOH in both male and female mice exposed to the CIE model. PNU120596 prevents the Ro 61-8048-induced reduction in EtOH consumption and preference. The Ro 61-8048-induced decrease in EtOH consumption and preference depends on the influx of KYN into the brain and is prolonged by maintaining the presence of KYN in the brain.

Conclusions

The inhibition of KMO and the consequent elevation in KYN reduces EtOH consumption and preference in both male and female in a model of dependence. This effect can be prevented by the allosteric modulation of α7nAChR, implicating these receptors in the effect of Ro 61-8048. This mechanism is centrally-mediated and the effect is maintained by blocking the efflux of KYN out of the brain. Thus, we demonstrate for the first time that the modulation of kynurenine pathway is a valid strategy for the treatment of alcohol dependence in both sexes.

Keywords

Ethanol, kynurenine, KMO, Ro 61-8048, Chronic intermittent ethanol (CIE)

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EFFECTS OF SYSTEMIC TREATMENT WITH GLYCINE AND LEUCINE-GLYCINE ON ACCUMBAL GLYCINE AND DOPAMINE LEVELS AND ETHANOL INTAKE IN THE MALE WISTAR RAT

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Objectives

Improved pharmacotherapy for Alcohol Use Disorder (AUD), aimed at the mechanisms by which ethanol interferes with the dopamine (DA) reward pathway, is warranted [1, 2]. Ethanol targets glycine receptors (GlyR) in the nucleus Accumbens (nAc), among other receptors [1, 3]. Local (into the nAc) and systemic treatment with glycine-transporter-1-inhibitors that modulate glycine levels and local perfusion with glycine itself, elevate basal and attenuate ethanol-induced nAc DA release and reduce ethanol intake in the rat [4-9]. Glycine treatment protocols in man require large doses and demonstrate variable brain glycine levels, probably due to impeded blood brain barrier (BBB) passage [10, 11]. Leucine-glycine (Leu-Gly), where glycine is anchored onto leucine that more readily passes the BBB, elevates whole brain tissue levels of DA in mice, measured ex vivo [12, 13]. In this study, the rational for elevating central glycine levels for reducing ethanol intake was further explored.

Materials and methods

The effects of glycine (200, 400, 800 mg/kg) or Leu-Gly (1, 10, 100, 1000) i.p. on nAc glycine and DA levels were examined using in vivo microdialysis in Wistar rats. The effects of the intermediate dose of glycine on voluntary ethanol intake and preference were examined in a limited access two-bottle ethanol/water model in the rat.

Results

Systemic glycine treatment increased nAc glycine levels in a dose-related manner whereas nAc DA levels were elevated in a subpopulation of animals, defined as DA responders. Ethanol intake and preference decreased after systemic glycine treatment. In contrast, Leu-Gly did not significantly alter nAc glycine or DA levels.

Conclusions

The results from the present study give further support to the concept of elevating central glycine levels to reduce ethanol intake and indicate that targeting the glycinergic system may represent a pharmacologic treatment principle for AUD.

Keywords

dopamine, alcohol consumption, in vivo microdialysis, glycine receptor, leucine-glycine, nucleus accumbens

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THE EFFECTIVENESS OF ELECTROCONVULSIVE THERAPY IN SUBSTANCE USE DISORDER AT PHARMACOLOGICAL TREATMENT FAILURE MAJOR DEPRESSION

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Objectives
Treatment resistant depression is common with Substance Use Disorder (SUD) and there are few studies that demonstrated the effectiveness of different medications in SUD related depression.

Materials and methods
All patients were diagnosed as treatment resistant depression with two trials of antidepressant medication failure and as a part of treatment program first completed the detox period and received at least two more antidepressant regimens for at least four months before they referred to ECT treatment. 14 patients 9 female 5 male (5 female and 2 male patients were addicted to prescription medications pain killers; benzodiazepines and-or sleeping pills) 2 female and one male patient alcohol and cannabis and two females and two male patients with polysubstance abuse disorder.

Results
The case series completed between 2011 to 2018 with follow up of 12.3+4.1 months after completing the ECT procedure. All patients received average 11.7+2.6 ECT treatments per series. Over 14 patients eleven of them responded well to ECT treatment at the first and intermediate phase of the follow up (first six months). Three patients did not received benefit from ECT treatment (two polysubstance use disorder and one alcohol use disorder). At intermediate to long term follow up period four patients reported relapse two of them renewed the substance abuse (one poly drug and the other is comorbid alcohol, cannabis addiction).

Conclusions
ECT treatment seems to be effective treatment for the patients with SUD & Depression. Moreover, the response rates are equal to treatment resistant depression cases without substance use disorder.

Keywords
Treatment resistant depression, SUD, ECT

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THE ROLE OF SEX AND GENETIC VARIATION IN A MOUSE MODEL OF FAS

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Objectives
Exposure to alcohol during early gestation is associated with craniofacial abnormalities, small eyes, and a wide range of neurological deficits. While genetics are a known mediator of alcohol sensitivity, the role of biological sex in determining the incidence and severity of alcohol-related birth defects is not fully understood. This study compares the effects of gastrulation-stage prenatal alcohol exposure (PAE) in male and female fetuses from several lines of genetically modified mice.

Materials and methods
For all studies, dams were treated with either alcohol (PAE) or vehicle on embryonic day (E) 7.0 and fetuses were observed for craniofacial defects on E17. In addition to C57BL/6J mice, we used mice with gene deletions in either p53 (apoptosis pathway), Htt (intracellular signaling), Kif3a (ciliary transport), or Efcab7 (Smo trafficking) on various background strains.

Results
In C57BL/6J mice, PAE females had a higher incidence of severe eye defects (48%) compared to males (30%). A similar effect was observed in the wild-type mice of all transgenic strains: PAE females had significantly more defects compared to males, independent of background strain. In some strains, an additional gene x sex interaction was observed. In the p53 mice, the protective effect of p53+/- and p53-/- was greatest in males. In the Htt strain, Htt+/- males were protected against a moderate alcohol dose and Htt+/- females were protected against a low alcohol dose. Conversely, Htt+/- females were more sensitive to moderate alcohol, indicating a possible sex x gene x dose interaction. In the Efcab7 or Kif3a mice, PAE females had more eye defects and craniofacial malformations compared to PAE males, regardless of genotype. Kif3a partial deletion did not affect sensitivity to PAE; full deletion of Efcab7 increased the severity of PAE-induced defects in both sexes.

Conclusions
Collectively, these data demonstrate that female mice are more sensitive to prenatal alcohol than males and that sex can interact with certain genotypes to impact outcome. Interestingly, the alcohol exposure in these studies is confined to the period of gastrulation, prior to sexual differentiation. Understanding how early gestational alcohol creates differential long-term outcomes in males and females is an important direction in the field of prenatal alcohol research.

Keywords
Fetal Alcohol Syndrome

References
None

UTILIZING 3D FACIAL ANALYSIS TO ESTIMATE THE PREVALENCE OF MINOR FACIAL ANOMALIES IN FASD

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Introduction: The facial gestalt of fetal alcohol syndrome (FAS) has long been established, with diagnosis heavily reliant on identifying the three cardinal features: a smooth philtrum; a thin upper lip; and a reduced palpebral fissure length (PFL). In addition, there are subtle, minor facial anomalies prevalent across the FASD spectrum, which are clinically challenging to distinguish and hence not utilized in clinical assessment. Current facial screening tools available to clinicians provide methods using 2D images require subjective assessment and identify only cardinal features. By employing 3D image analysis, we can objectively identify and measure subtle facial dysmorphism, estimate the prevalence of minor anomalies across the FASD population and provide beneficial clinical feedback on individual facial assessment.

Material and Methods: A Caucasian and African American cohort of participants aged 3 to 18 years was recruited by the CIFASD consortium. Participants underwent full dysmorphology examinations accompanied by facial images acquired using a high-resolution 3D cameras. For each individual, dense surface modelling analysis produced facial heat maps of normalised differences delineating facial dysmorphism. Individual automated anthropometric measurements, and a volumetric measure of the mandible representing micrognathia were calculated to define clinically relevant metrics.

Results: We observe a plethora of facial dysmorphism across the FASD spectrum. In particular, midfacial hypoplasia, micrognathia and shape differences on the nose are observed in a significantly greater number of individuals with alcohol exposure compared to controls. The prevalence of these minor anomalies appear dose dependent in both ethnic groups. The techniques used are capable of detecting subtle facial differences which may provide a robust method for assessing facial dysmorphism in FASD.

Conclusions: Assessment of subtle facial features associated with prenatal alcohol exposure is often overlooked, and is particularly challenging when an individual lacks criteria for a FAS diagnosis. Our techniques provide a surface-based analysis of facial dysmorphia utilising the precision of 3D imaging. Emerging 3D camera technologies based on smartphone and tablet will reduce the cost and complexity of 3D imaging and hence provide a viable option for future placement in FASD clinics.

Keywords: FASD, Facial Dysmorphism, Prenatal Alcohol Exposure, 3D Facial Analysis

WHAT ROLE FOR PROTECTIVE BEHAVIORAL STRATEGIES: MODERATION OR MEDIATION OF PSYCHOLOGICAL DETERMINANTS ASSOCIATED WITH BINGE DRINKING AMONG UNIVERSITY STUDENTS?

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Objectives

Binge Drinking (BD), a heavy alcohol consumption over a short period (NIAAA, 2004) is a major public health issue among university students (Tavolacci et al., 2016). Research evidenced several psychological determinants of BD (Mange et al., 2021), and, to a lesser extent, the use of alcohol Protective Behavioral Strategies (PBSs). These PBSs are specific behaviors used to minimize the harmful consequences of alcohol consumption, and seem to stand out from the other psychological determinants since they can either moderate (Weaver et al., 2012) or mediate (Bravo et al., 2017) the effects of these determinants on alcohol outcomes. While these moderating and mediating roles of PBSs are quite clear in the alcohol context, they remain little explored in the specific BD practice. Therefore, in an integrative model, this study aims to test the moderating and mediating roles of PBSs on different psychological determinants of BD among students.

Materials and methods

Within the ADUC project (https://www.researchgate.net/project/ADUC-Alcool-et-Drogues-a-lUniversite-de-Caen-Normandie-Alcohol-and-Drugs-in-University-of-Caen-Normandy),1944 university students (Mage = 19.9; SD = 2.19; 64.4% of women) completed online questionnaires which assessed alcohol-related variables (AUDIT, BD score), demographics (sex, age), psychological factors (norm, drinking identity, drinking motives, meta-cognitions, impulsivity and personality traits) and three PBSs (limiting/stopping drinking, manner of drinking and harm reduction). A statistic model then allowed to test both the PBSs moderating (Determinants X PBSs-subscales BD) and mediating roles (Determinants PBSs-subscales BD), using percentile bootstrapped estimates (95% CI, N = 10,000; Yzerbyt et al., 2018).

Results

The role of PBSs differs according to their type. First, the use of PBS-limiting only moderates determinants' effects by weakening the effects of uncontrollability and lack of premeditation on BD. Second, the use of PBS-harm-reduction is both a moderator by weakening the effects of age, drinking identity, lack of premeditation and perseveration, but also a mediator of the effect of sex on BD (17.1%). Third, the use of PBS-manner only has a mediating role on age (100%), anxiety (100%), sensation seeking (100%), cognitive self-regulation (100%), emotional self-regulation (23.4%), social motive (23.0%), lack of premeditation (18.3%), sex (15.3%) and enhancement motive (15.8%).

Conclusions

Considering PBSs as multidimensional constructs have implications both at empirical level, by deepening the conditions under which psychological determinants impact BD, and at a prevention level, to develop adapted strategies aiming at reducing BD.

Keywords

Binge drinking, protective behavioral strategies, psychological determinants, moderation/mediation, university students

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ALCOHOL USE AND BINGE DRINKING AMONG FIRST-YEAR UNIVERSITY STUDENTS: RISK FACTORS AND THE COVID-19 HEALTH CRISIS EFFECT

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Objectives

Heavy alcohol use is considered as a major public health issue[1]. Several risks factors have been identified, especially anxiety and depressive disorders. Yet, we know that these psychological disorders (1) have increased during the COVID-19 pandemic[2] and (2) are more prominent during the transition to adulthood like a first-year university[3]. Therefore, we aimed to identify the prevalence and risk factors of alcohol use disorders (AUD) among first-year university students both before and during the COVID-19 pandemic.

Materials and methods

Within the ADUC project (https://www.researchgate.net/project/ADUC-Alcool-et-Drogues-a-lUniversite-de-Caen-Normandie-Alcohol-and-Drugs-in-University-of-Caen-Normandy), 2247 first-year students completed an annual online survey between 2017 and 2020 measuring substances use variables, sociodemographic and psychological factors. To test the impact of COVID-19 pandemic on consumption, participants in years 2017-2019 were compared to participants in years 2020. Multinomial and binomial logistic regression models were performed.

Results

Regardless the COVID-19 pandemic, men were more likely to have severe AUD and to practice BD, smoking increases risks of moderate and severe AUD and BD, and depression increases the risk of severe AUD. Living with parents is a protective factor against severe AUD and BD. During the COVID-19 period, students were not more anxious and depressed than students in 2017-2019 years, and were less likely to use alcohol and to practice BD. Students who continued to drink were more likely to present severe AUD.

Conclusions

Our study highlights some common risk factors for substance use disorders among first-year university students prior to the COVID-19 period. Furthermore, while the health crisis appears to have been an additional risk factor for some user profiles, it may also have been a protective factor for others. The challenge of conducting future research on these different profiles will be discussed.

Keywords

alcohol use disorder, binge drinking, risk factors, COVID-19.

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CANNABINOIDS MODULATE COGNITIVE DEFICITS AND NEUROINFLAMMATION INDUCED BY EARLY ALCOHOL EXPOSURE.

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Objectives

Foetal alcohol spectrum disorder (FASD) is the term used to describe the physical, mental and behavioural disabilities induced by prenatal and lactation alcohol exposure (PLAE). Numerous molecular mechanisms might be underlying the alcohol-induced teratogenicity, including neuroinflammatory reactions and the alterations of the endocannabinoid system. In this regards, cannabidiol modulates endocannabinoid system and also appears to produce anti-inflammatory effects. In addition, the role of endocannabinoids in the modulation of neuroinflammation has been well documented, acting on different targets, including the PPAR system. In this context, our study aims i) to assess whether cannabidiol could ameliorate cognitive impairments in PLAE mice and ii) to study whether the interaction between endocannabinoids and PPAR system might induce anti-inflammatory effects that would regulate cognitive impairment in FASD-like mouse model.

Materials and methods

For that, to achieve the first objective, we used a model of alcohol binge drinking during gestational and lactation periods in pregnant C57BL/6 female mice (1). Following the prenatal and lactation alcohol exposure, we treated the male and female offspring with cannabidiol from post-natal day (PD) 25 until PD34, and we evaluated their cognitive performance at PD60 (2). For the second objective, we used the same procedure, and we treated offspring with the FAAH inhibitor URB597 and the PPARγ antagonist GW9662 during the same period (PD25 to PD34), and then at PD60, we evaluated cognitive performance and pro-inflammatory markers.

Results

Our results showed that cannabidiol and URB597 treatment during peri-adolescence period ameliorates cognitive deficits observed in our FASD-like mouse model, without sex differences. Moreover, the beneficial effects of URB597 were prevented by the treatment with the PPAR antagonist. Additionally, cannabidiol restored levels of TNFα and IL-6 in the hippocampus.

Conclusions

In conclusion, our study provides new findings about the participation of endocannabinoid system in behavioural and molecular pathological mechanism underlying FASD. Our data also suggests that cannabidiol could represent a therapeutic agent to counteract cognitive impairments and neuroinflammation in FASD.

Keywords

FASD, cannabidiol, endocannabinoids, inflammation, PPARγ

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CHRONIC ETHANOL EXPOSURE PRODUCES ERECTILE DYSFUNCTION BY IMPAIRING THE NO-CGMP PATHWAY

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Objectives

Introduction Erectile dysfunction is defined as the consistent or recurrent inability to attain and/or maintain penile erection sufficient for sexual satisfaction [1]. Penile erection is a complex neurovascular process where relaxation of the corpus cavernosum smooth muscle is essential for normal erectile function [2]. Chronic ethanol consumption has been related to a higher risk of suffering from erectile dysfunction [3]. Some studies have reported a prevalence of erectile dysfunction around 60% in alcoholic patients [4]. However, the molecular mechanisms underlying the erectile dysfunction caused by ethanol consumption are not yet fully understood.

Materials and methods

Adult male C57BL/6J mice were subjected to the Chronic Intermittent Ethanol (CIE) paradigm, an ethanol dependence and relapse drinking model [6]. On the last day of CIE, mice were sacrificed, the penises were removed and corpus cavernosa dissected out. Mice corpus cavernosa were mounted in a myograph with Krebs buffer solution maintained at 37 °C and bubbled with 95% O2 and 5% CO2. Relaxation to electrical field stimulation, acetylcholine, riociguat (a stimulator of cGMP route, whose action is dependent on the oxidation state of soluble guanylate cyclase (sGC)), cinaciguat (an activator of cGMP route, whose action is independent on the oxidation state of sGC) and forskolin (an activator of the cAMP route) were studied.

Results

CIE exposure produced a significant decrease in the relaxant response of the corpus cavernosa to electrical field stimulation, acetylcholine and riociguat. Conversely, the response to cinaciguat was significantly improved in these corpus cavernosa. No differences were observed in the response to forskolin between control and ethanol-dependent mice.

Conclusions

Chronic ethanol exposure produces in vitro erectile dysfunction via dysregulation of the NO-cGMP pathway. The relaxation via cAMP remains intact. The decrease in the potency of riociguat and the increase in the potency of cinaciguat indicate that CIE exposure alters the redox state of sGC. cGMP activators are potential drugs for treating erectile dysfunction in alcoholic patients.

Keywords

Chronic Ethanol exposure, erectile dysfunction, soluble guanylate cyclase

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NEURAL CORRELATES OF RECALLING OF ALCOHOL CONSUMPTION CONTEXTS IN YOUNG BINGE DRINKERS: AN FMRI STUDY DURING AN ASSOCIATIVE MEMORY TASK

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Objectives

Binge drinking (BD) is defined as a pattern of high alcohol intake in a short time followed by periods of abstinence (NIAAA, 2015). This behaviour is very common during adolescence and early adulthood, a developmental stage characterized by the maturation of prefrontal and striatal networks, important circuits related to the capacity to control and reinforce behaviours (Crews et al., 2007). The neural mechanisms underlying binge drinking are unclear, but recent studies have reported that binge drinkers (BDs), similar to addicted, had greater cue-elicited brain response in this network, including dorsal striatum, parahippocampal gyrus, cerebellum and thalamus (Dager et al., 2014; Brumback et al., 2015).In the present functional magnetic resonance imaging (fMRI) study, we investigate the presence of neurofunctional anomalies involved in reward and memory processes when young binge drinkers (BDs) are exposed to visual stimuli previously associated with drinking contexts.

Materials and methods

Twenty college BDs (10 women) between 18-23 years old and 16 age-matched abstainers (10 women) underwent a fMRI acquisition during the performance of an associative memory task. One day prior to scanning participants studied 90 images of objects with neutral emotional valence paired with 90 images of young people in two different social settings: alcohol drinking and reading/studying contexts. During the scanning, participants made memory judgments about the context (alcohol drinking or reading/study) that was associated with the object image presented. Whole brain analyses of the functional images were performed using SPM12. Voxel-wise threshold of p

Results

BDs showed increased BOLD signal during the recall of objects associated with alcohol consumption contexts compared to abstainers in the cerebellum, occipital cortex, thalamus and globus pallidus.

Conclusions

These findings are in line with results from recent neuroimaging studies (Brumback et al., 2015; Campanella et al., 2017; Fede et al., 2019;) and suggest a pattern of hyperactivation in visual action-driven and incentive/emotional salience regions in young BDs during the recall of alcohol drinking contexts, which might be related to the craving and impulsive behaviours that characterize BD.

Keywords

Binge drinking, fMRI, College Students, Associated Memory, Alcohol.

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CHRONIC ETHANOL INTAKE INFLUENCES GENE EXPRESSION OF SARS-COV2 INFECTION-RELEVANT GENES IN AN ORGAN-SPECIFIC MANNER

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Objectives

Since the end of 2019, the severe acute respiratory syndrome Corona-virus 2 (SARS-CoV2) has caused several millions of infections and deaths worldwide. It has been reported that certain risk factors like chronic lung disease, autoimmune dysfunction and diabetes are promoting SARS-CoV2 infection and worsening the course of disease. There is some evidence suggesting that chronic alcohol consumption might have an impact on SARS-CoV2 infection risk, but referring to this, there is little molecular data published so far. In this study, we obtained gene expression data following acute and chronic alcohol intake in the context of genes that are known to be involved in SARS-CoV2 infection. We hypothesize, that long-term alcohol intake causes a change in gene expression of SARS-CoV2 infection-relevant genes.

Materials and methods

We used three different animal models of chronic ethanol intake – repeated intermittent Ethanol IP injections, vapor exposure for seven weeks, and the post-dependent model - and measured gene expression of Ace2, Tmprss2 and Mas by qPCR in six different organs: lung, heart, liver, kidney, ileum, and brain. ACE2 and TMPRSS2 represent the virus entry point, whereas Mas is activating the anti-inflammatory response, once the cells are infected.

Results

Across the three animal models of chronic alcohol consumption, we found an organ-specific up-regulation of all three genes of interest. In the brain, Mas was down-regulated in both human postmortem and rat brain tissue, while Ace2 was too little expressed to obtain meaningful results.

Conclusions

This comparative study of three different animal models of chronic ethanol intake suggests that long-term ethanol intake might consistently up-regulate gene expression of SARS-CoV2 infection-relevant genes in an organ-specific manner. An up-regulated Ace2 gene expression might lead to an elevated stochastic probability of virus entry, but also to an enhanced anti-inflammatory response via the ACE2/Ang(1-7)/Mas axis.

Keywords

Alcohol, SARS-CoV2, ACE2, gene expression, mRNA

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BRAIN FUNCTIONAL HYPERCONNECTIVITY DURING PROCESSING OF ALCOHOL-RELATED IMAGES IN YOUNG BINGE DRINKERS

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Objectives

Alcohol attentional bias has been pointed as a major marker of alcohol misuse (Field and Cox, 2008). Recent evidence has showed that brain functional connectivity (FC) may be a valuable index of the brain networks' integrity in young binge drinkers (BDs) (Arienzo et al., 2020; Sousa et al., 2019). Nevertheless, despite the major implications that attentional bias may have on alcohol-use behavior and craving, to the best of our knowledge, there is no study to date examining the FC networks linked to the processing of alcohol-related images in this population. Therefore, the present study aimed to explore the FC signatures underlying attentional bias toward alcohol stimuli in young BDs.

Materials and methods

Electroencephalographic (EEG) activity was recorded in 54 college students (55.5% females; 27 non/low-drinkers and 27 BDs) while performing a visual alcohol cue-reactivity task. We evaluated whole-brain FC profiles during the processing of alcohol and non-alcohol cues, as well as their potential relationship with craving and severity of alcohol use.

Results

At the behavioral level, BDs rated alcohol-related images as more pleasant/attractive than non/low-drinkers. Furthermore, at the electrophysiological level, BDs exhibited increased beta-band FC – particularly in the fronto-parieto-occipital network- when processing alcoholic cues. Conversely, they displayed reduced theta-band FC relatively to non/low-drinkers for non-alcoholic images. These hyper-/hypo-connectivity patterns were associated with more harmful alcohol use and higher alcohol craving levels.

Conclusions

Findings are congruent with previous neurofunctional studies reporting an attentional bias towards alcohol-related information in BDs (Almeida-Antunes et al., 2021; Brumback et al., 2015). These results may have important clinical implications as this neural reactivity to alcoholic cues may contribute to the maintenance and/or escalation of the drinking pattern. Finally, the present study constitutes the first evidence showing that FC networks may be a sensitive indicator to alcohol attentional bias in BDs.

Keywords

binge drinking, attentional bias, alcohol images, alcohol cue reactivity, electroencephalography, functional connectivity

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FOLLOW-UP OF TRANSPLANT PATIENTS FOR HEPATITIS ACUTE ALCOHOLIC AND RELAPSE ASSESSMENT

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Objectives

Acute alcoholic hepatitis is responsible for 10% of hepatic disease mortality alcoholics. Despite treatment with corticosteroid therapy in severe forms the risk of mortality at 6 months in case of failure is greater than 70%. The benefit of a transplant early survival in these patients has been proven. Before the shortage of grafts, it is essential to select the patients with minimal risk of relapse. This assessment is limited by the absence of prior weaning in transplant patients for acute alcoholic hepatitis. The principal objective was to assess the relapse rate of patients. The secondary objectives were to put evidence of possible relapse factors and assess the survival at 1 month, 1 year and 5 years. The occurrence of complications serious in the first postoperative month defined by a organ failure or emergency surgery has was analyzed as well as their predictive risk factor.

Materials and methods

This is a study observational on a historical cohort prospective patients were asked to present a clinico-biological compatible with alcoholic hepatitis severe acute and have been transplanted between 01/01/2013 and 01/01/2020 +. The transplant patients after 6 months of effective alcohol withdrawal were excluded Any alcohol consumption after the transplant was counted as a relapse. This was evaluated by a questionnaire given to the patient. For those who could not answer the questionnaire the relapse was assessed on data from previous consultations.

Results

General characteristics and methods of transplantation: 20 patients were included including 75% of men with a median age of 58 years. Maddrey's median score was 78. A transfer to resuscitation before the transplant was necessary in 60% of case and median MELD score on transplant day was 31. Alcohol relapse: At 1 year the relapse rate was 5% and 25% (n = 5/20) over the total duration of the study with a median 3-year follow-up. Relapse occurred with a median delay 48 months. No epidemiological, psycho-social or concerning alcohol consumption before transplantation was not predictive of relapse. For the 4 relapsers including us had the questionnaire consumption did not exceed 30 grams per day and was mostly occasional. Only one case weaned his consumption. All patients had spoken of their relapse either to their entourage or to their doctor but none had wanted addiction treatment specific. Survival and Complication: The survival rate at one month of transplant is 95%, 1 year 90% and 5 years 85%. From serious early complications occurred in 50% of cases: 20% of cases presented with organ failure including 10% of septic shock and 25% had a surgical revision. No retransplantation was necessary. Only the quantity of red blood cells transfused during the transplant emerged as a risk factor for complications.

Conclusions

Our study analyzes a patient population transplant recipients for acute alcoholic hepatitis. the 1-year relapse rate is less than 10% and amounts to a total of 25% over a median follow-up of 3 years, i.e. equal to that of patients transplant recipients for alcoholic cirrhosis. Consumption was mostly occasional and did not exceed 30 grams of alcohol. Despite a high 50% number of early complications the 5-year survival rate is 85%. Only the quantity of blood cells transfused during the procedure appeared to be predictive of early complications.

Keywords

Acute alcoholic hepatitis

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SCREENING FOR PORTAL HYPERTENSION ACCORDING TOBAVENO VI RECOMMENDATIONS

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Objectives
From the Baveno recommendations VI of 2015, screening for portal hypertension (PH) by eso-gastro-duodenal endoscopy (EOGD) is not more systematic but only recommended for patients with decompensated cirrhosis or with a rate of platelets ≤ 150 G / L and / or elastometry ≥ 20 kPa. These recommendations were validated in a cohort patients with chronic viral hepatitis but their extrapolation to patients with non-viral cirrhosis has not been validated so far. Excessive alcohol consumption being the main cause of cirrhosis in Romania. This study had for the purpose of evaluating the validation of these recommendations in a cohort of patients with compensated alcoholic cirrhosis.

Materials and methods
Patients with histologically proven alcoholic cirrhosis and compensated (Child-Pugh A) were selected for this study if a FOGD, a blood platelet test and a elastometry performed at inclusion were jointly available. Patients were excluded if elastometry was uninterpretable (IQR> 30% of the median) or if they had a history of gastrointestinal bleeding by PH or ligation endoscopic of esophageal varices (VO). The criterion of main judgment was the presence of large VO at screening EOGD or during follow-up (grade II or III). The progression of PH was defined by the appearance of VO of large size or the need for endoscopic ligation (a all the more so during a bleeding episode) during follow-up.

Results
20 patients were selected for the initial evaluation study : male 75%, median age 53.9 years, median body mass index 29.2 kg / m², persistence 40% regular alcohol consumption. Including, 10 patients had a platelet count> 150 G / L and a elastometry 110 G / L and elastometry 110 G / L and elastometry 150 G / L and a elastometry

Conclusions
The screening EOGD can indeed be avoided in patients with alcoholic cirrhosis compensated, platelet count> 150 G / L and elastometry < 20 kPa, due to a low risk of large size OV, in accordance with the recommendations of Baveno VI. However, the presence of metabolic liver disease cofactors is a risk factor for worsening PH and is probably lead to more specific monitoring.

Keywords
Excessive alcohol consumption, portal hypertension, Baveno VI recommendations

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Assessing portal hypertension in liver diseases Annalisa Berzigotti
https://www.tandfonline.com/doi/full/10.1586/egh.12.83

THE OCCURRENCE OF PNEUMONIA IS A MAJOR PROGNOSTIC ELEMENT IN SEVERE ALCOHOLIC HEPATITIS TREATED WITH CORTICOSTEROIDS

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Objectives

The risk of infection is high in patients with severe alcoholic hepatitis (1, 2) treated with corticosteroids, in particular with frequent occurrence of pneumonia (1, 3). Development of infection appears to be associated with poorer survival. However, the prognostic risk of the presence of lung disease has not been specifically analyzed in a dedicated cohort. The aim of this work was to assess the impact of the occurrence of pneumonia before, during and after the start of corticosteroid therapy (compared to other commonly observed infections) on the 2-month survival of patients with severe HA treated with corticosteroids.

Materials and methods

All the patients admitted to our unit for severe HA (Maddrey score ≥ 32) between 2016 and 2020 were included prospectively, with a large and systematic infectious assessment on admission. This assessment was repeated if infection was suspected during follow-up. Oral corticosteroid therapy was started in the absence of contraindications, particularly infectious ones, the therapeutic response was defined by the Lille score calculated on the seventh day.

Results

60 patients were included, of whom 20 (33.3%) had an infection on admission including 15 pneumonia (4 pneumocystoses). In multivariate analysis, the presence of encephalopathy (OR 1.64, 95% CI 1.05-2.57, p = 0.03), the MELD score (OR 1.09, 95% CI 1.04- 1.15, p = 0.0003) and active or former smoking (OR 3.77, 95% CI 1.69-8.4, p = 0.001) were independently associated with the risk of developing lung disease on admission (respective p

Conclusions

The occurrence of pulmonary infection in patients with severe alcoholic hepatitis is common and is an independent determinant of mortality upon admission to the patient and after initiation of corticosteroids. This underlines the importance of promptly diagnosing and treating pulmonary infectious events and raises the question of systematic evaluation by chest CT before and after treatment.

Keywords

Pneumonia, alcoholic hepatitis

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NEUROPSYCHOLOGY OF ADDICTION IN THERAPEUTIC COMMUNITIES: A SPECIFIC COGNITIVE SEMIOLOGY

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Objectives
Therapeutic Communities (TCs) are care units welcoming people with Alcohol and Drug Use Disorder (AUD/DUD) and frequent psychiatric comorbidities. Those alterations can lead to neuropsychological impairments which are likely to hinder the benefit of addiction treatment. However, these disorders are rarely considered in the support of TCs residents. The aims of this study are therefore to describe (1) the consumption profile of the residents and (2) their neuropsychological impairment profile.

Materials and methods
Residents of 3 French TCs underwent clinical interviews and questionnaires (health, psychiatric history, DSM-V criteria for substance use, consumption data) and a neuropsychological screening (the BEARNI; Ritz et al., 2015). The sample of TCs residents (n=26) was compared to a healthy control (HC) group (n=26) matched for age, gender and education.

Results
All TCs residents had psychiatric comorbidities, AUD and used tobacco. Most of them were polyconsumers, with 80% using cannabis and 65% cocaine among the most frequent substances used. Regarding cognition, HC performed better than all TCs residents on ataxia, working memory, episodic memory and visuospatial abilities subtests as well as BEARNI total score, but no difference was found on flexibility.

Conclusions
Results indicate a heterogeneous consumption profile in TCs residents, with polyconsumption. TCs residents were globally cognitively impaired compared to HC. This data is a first step in better understanding specific TCs residents' consumption and cognitive profiles. It highlights the need to adapt care and treatment in TCs resident who may be at-risk of neuropsychological impairments to support the benefit of addiction care in TCs.

Keywords
Neuropsychology, Alcohol Use Disorder, drugs, therapeutic communities, addiction treatment

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NEUROIMMUNITY AND MU-OPIOID RECEPTORS: IMPLICATIONS FOR PAIN-INDUCED ALCOHOL RELAPSE

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Objectives

There is evidence concerning the role of alcohol-induced neuroinflammation in alcohol intake and relapse (1-3). Moreover, Mu-Opioid Receptors (MORs) mediate the reinforcing properties of alcohol (4) and, interestingly, previous research suggests that neuroinflammation and MORs could be related (5-7). Our objective is to study neuroinflammatory states and microglial activation, together with adaptations on MORs expression in the mesocorticolimbic system during abstinence and relapse phases.

Materials and methods

We have used a sex-dependent rat model of complete Freund's Adjuvant (CFA)-induced alcohol deprivation effect (ADE). Then the biochemical tools used were immunohistochemistry in order to assess microglial activation and western blot to analyze MORs and neuroinflammatory mediatios,

Results

Firstly, our results confirm that only CFA-treated female rats, the only experimental group that showed relapse-like behavior, exhibited specific alterations in the expression of phosphorylated NFκB, iNOS and COX2 in the PFC and VTA. More interestingly, the analysis of the IBA1 expression revealed a decrease of the microglial activation in PFC during abstinence and an increase of its expression in the relapse phase, together with an augmentation of this activation in the NAc in both phases that only occur in female CFA-treated rats. Additionally, the expression of IL1β also evidenced these dynamic changes through these two phases following similar expression patterns in both areas. Furthermore, the expression of the cytokine IL10 showed a different profile than that of IL1β, indicating anti-inflammatory processes occurring only during abstinence in the PFC of CFA-female rats but not during the re-introduction phase in PFC nor in the NAc. This data indicates a downregulation of microglial activation and pro-inflammatory processes during abstinence in the PFC, whereas an upregulation can be observed in the NAc during abstinence that is maintained during the re-introduction phase only in CFA-female rats. Secondly, our data reveals a correlation between the alterations observed in IL1β, IBA1 levels and MOR levels in the PFC and NAc of CFA-treated female rats.

Conclusions

Although premature, our data suggests that neuroinflammatory processes, together with neural adaptations involving MOR, might play an important role in alcohol relapse in female rats, so further investigations are warranted.

Keywords

mu-opioid receptor, alcohol, Pain, alcohol deprivation effect, Microglia, Neuroinflammation

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THE CHARACTERISTICS OF ALCOHOL RECOVERY STORIES SYSTEMATIC REVIEW AND NARRATIVE SYNTHESIS

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Objectives
Alcohol recovery narratives have been collected in research studies, but no overarching conceptual framework exists. We aim to systematic review literature to develop a framework of over-arching narrative typologies (structures) and themes (content) characterizing alcohol recovery narratives to inform the development of future research, policy, and practice within healthcare and other settings.

Materials and methods
Electronic searches were conducted using Ovid MEDLINE, EMBASE, CINHAL, PsychInfo, AMED and SCOPUS (inception- March 2021) along with additional techniques (e.g., Google Scholar, ProQuest, Clinical Trials). Alcohol recovery narratives were defined as "first-person lived experience accounts, which include elements of adversity, struggle, strength, success, and survival related to alcohol misuse, and refer to events or actions over a period". Studies including participants presenting or advancing an original framework of typologies and/or themes of alcohol recovery narratives were included. Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidance was followed. Protocol was registered on Prospero (CRD42021235176)

Results
A total 32 papers were included in the review (29 qualitative, 3 mixed methods; N=1055 participants, age range 17-82 years, 52.6% male). Most studies were conducted in the United States (n=15) and Europe (n=11). No recovery narratives were found from lower-middle income countries. Treatment settings included Alcoholic Anonymous (AA, n=12 studies), other formal treatment, and 'natural recovery'. Thematic analysis resulted in eight dimensions (genre, identity, recovery setting, drinking trajectory, drinking behaviours, stages, spirituality and religion, and recovery experience) arranged in three superordinate categories (form, structure, and content). A subgroup analysis showed all dimensions were present in most subgroups, although shame was more prominent in female only narratives, lack of sense of belonging and spirituality in homosexual narratives, and alienation and inequality in native Alaskan and Aboriginal narratives.

Conclusions
Results highlight a significant gap for alcohol recovery narratives from narrators not in the United States or Europe, or those following the AA model of recovery. The review provides a framework for the development of the broad and inclusive definition of recovery narratives.

Keywords
Alcoholism. Alcohol-related disorders. Alcohol misuse. Narration. Alcohol recovery story. Recovery narratives. Systematic review.

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MULTI-OMICS SIGNATURES OF ALCOHOL USE DISORDER IN THE VENTRAL AND DORSAL STRIATUM

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Objectives

Alcohol Use Disorder (AUD) is a major contributor to global mortality and morbidity. The analysis of postmortem human brain tissue enables to investigate molecular mechanisms associated with AUD in brain regions. This study aimed to identify differentially expressed (DE) genes in the ventral and dorsal striatum between individuals with AUD and controls, and to integrate the results with findings from genome- and epigenome-wide association studies to identify functionally relevant molecular mechanisms of AUD.

Materials and methods

DNA-methylation and gene expression (RNA-seq) data was generated from human postmortem brain samples of 48 individuals with AUD and 51 controls from the ventral striatum (VS) and the dorsal striatal regions caudate nucleus (CN) and putamen (PUT). We identified DE genes using DESeq2, performed gene-set enrichment analysis (GSEA) with fgsea, and tested enrichment of DE genes in results of genome-wide association studies (GWAS) using MAGMA. Weighted correlation network

analysis (WGCNA) was performed for both DNA-methylation and gene expression data and gene overlap was tested.

Results
Results showed DE genes at FDR < 0.05 in the dorsal striatum. In the VS, results at FDR < .25 were overrepresented in a recent GWAS of problematic alcohol use. ARHGEF15 was upregulated in all three brain regions. GSEA in CN and VS results pointed towards cell-structure associated GO-terms and in PUT towards immune pathways. The WGCNA modules most strongly associated with AUD showed strong enrichment for immune response and inflammation pathways

Conclusions
Our integrated analysis of multi-omics data sets provides further evidence for the importance of immune-and inflammation-related processes in AUD.

Keywords
RNA-seq, genome-wide, gene expression, DNA-methylation, neuroinflamamtion

References
No References

ADVANCED NETWORK ANALYSIS OF CFOS RESPONSES REVEALS DISTINCT BRAIN STATES FOR ALCOHOL AND SWEET MEMORY RECALL

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Objectives
Cue-reward associations form distinct memories that can drive appetitive behaviors and cravings for both drugs and natural rewards. It is still unclear how such memories are encoded in the brain's reward system.

Materials and methods
We trained rats to concurrently self-administer either alcohol or a sweet saccharin solution as drug or natural rewards, respectively. Memory recall due to cue exposure reactivated reward-associated functional ensembles in reward-related brain regions, marked by a neural cFos response. As shown in a previous study the local ensembles activated by cue presentation for either reward consisted of similar numbers of neurons [1]. Here we used advanced statistical network analysis and we found robust reward-specific co-activation patterns across brain regions.

Results
Interestingly, the resulting meta-ensemble networks differed in several things: 1) the connectivity strength and edge-weight distributions, 2) the modular structure of the functional network, which seems to be strongly affected by alcohol; 3) communication efficiency in the network is significantly smaller in case of alcohol; 4) and the most influential regions, which in case of saccharin comprised the prefrontal cortex, while for alcohol seeking control shifted to insular cortex with strong involvement of the amygdala.

Conclusions
Our results support the view of memory representation as a differential co-activation of local neuronal ensembles [2].

Keywords
Network analysis Reward seeking Prefrontal cortex Insular cortex Neuronal ensembles

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DNA METHYLATION-BASED ASSESSMENT OF SMOKING STATUS IN THE BRAIN

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Objectives
Long-term smoking can lead to the development of nicotine dependence, and accumulating evidence points towards the involvement of epigenetic mechanisms in the etiology of substance use disorders. Whereas characteristic methylation signatures of smoking have been identified in tissues such as blood or lung, there is only limited knowledge on the effect of smoking on DNA methylation levels in the brain. As self-reports and measurement of blood nicotine levels have major limitations, blood methylation-based prediction algorithms have been developed for assessing smoking status. However, it is still unclear, to what degree blood derived marker CpG sites are also applicable for smoking status estimation using brain tissue. Also, there is only few information on tissue specificity of smoking-associated methylation signatures. One recent study in human post-mortem nucleus accumbens tissue indicated distinct smoking-associated CpG sites for brain tissue in comparison to blood.

Materials and methods
We tested DNA methylation signatures of smoking using recently generated epigenome-wide methylation data (Illumina HumanMethylationEPIC Beadchip) from multiple brain regions of n=80 subjects (38 healthy controls, 42 with alcohol use disorder (AUD)). In total, five brain regions implicated in the neurocircuitry of addiction were investigated with adjustment for AUD: anterior cingulate cortex, Brodmann Area 9, caudate nucleus, putamen, and ventral striatum. For an additional n=11 independent subjects (6 healthy controls, 5 with AUD), matched samples of Brodmann Area 9 and blood were analyzed enabling direct comparison of methylomes between tissues. A multi-marker prediction algorithm developed on the basis of blood methylation data was used to estimate smoking status in the brain.

Results
Using the multi-marker prediction method, stable associations of smoking status with DNA methylation in brain and blood were identified. When applied to the different brain regions, smokers were reliably differentiated from non-smokers.

Conclusions
Our results indicate an overlap of smoking-associated methylation signatures in blood and different brain regions. Predictive CpG sites for smoking in blood could thus also serve for the estimation of smoking status in the brain. More refined analyses will be performed to identify the CpG sites most reliably associated with smoking across different tissues.

Keywords
Smoking, EWAS, methylation

References
No references.

PATIENT DERIVED PRECISION-CUT LIVER SLICES AS A VERSATILE IMMUNOCOMPETENT PLATFORM TO STUDY NOVEL THERAPEUTICS FOR ALCOHOL-RELATED LIVER DISEASE

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Objectives
Alcohol-related Liver Disease (ALD) encompasses several aetiopathological characteristics including steatosis, fibrogenesis, cell death, inflammation, metabolic alterations and mitochondrial dysfunction. The canonical experimental models present significant limitations in recapitulating these complex disease processes and these hinder both the identification of novel therapeutically targetable pathways and the preclinical evaluation of emerging drugs. Here we present the development of an immunocompetent ex-vivo model of ALD, based on the culture of human Precision-Cut Liver Slices (PCLS)(1), and its direct application for the assessment of the therapeutic effects of the cyclophilin inhibitor, CRV431 (2).

Materials and methods
PCLS were prepared from human tumour-free liver specimens (at different fibrotic stages) from patients (n=8) undergoing resection of liver metastasis. PCLS were exposed to hepatotoxic insults (ethanol 250mM, oleic/linoleic acids 0.1mM, LPS 10µg/ml) for up to 5 days (3). The effect of 5µM CRV431 was studied for the duration of the culture. Cell viability/organelle functionality was evaluated by histology, ATP content, cytokeratin-18 release and mitochondrial assays. Fibrosis was assessed by gene expression and secretion of fibrotic markers. Pro-inflammatory cytokines were quantified by Luminex.

Results
The exposure of PCLS to ethanol and other insults for increasing time periods mimicked the development of key ALD features, namely steatosis, mitochondrial alteration, inflammation and fibrosis. PCLS viability was confirmed by histology and ATP content throughout the culture and the increased activation of fibrogenic pathways was observed over time. The expression and secretion of fibrotic and pro-inflammatory markers were exacerbated in the PCLS treated with the insults. CRV431 treatment was well tolerated in the PCLS, and no hepatotoxic effects were observed. In addition, the drug notably reduced the expression of fibrotic markers in PCLS that originated from fibrotic liver tissue particularly when challenged with the hepatotoxic insults ex-vivo. Finally, CRV431 restored a balanced cytokine profile in PCLS exposed to LPS.

Conclusions

In summary, this model has successfully recapitulated a spectrum of pathologies associated with the progression of ALD and is validated for pre-clinical studies to test the efficacy of immunomodulatory and anti-fibrotic drugs. Furthermore, our results have shown for the first time the potential of cyclophilin inhibitor CRV431 in ALD.

Keywords

Alcohol-related Liver Disease, Precision-cut liver slices, ex vivo models, cyclophilin inhibitor, fibrosis

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TEMPORAL DYNAMICS OF IMPULSIVITY AND CRAVING BEFORE AND AFTER A BINGE DRINKING EPISODE: RESULTS FROM AN ONGOING EXPERIENCE SAMPLING STUDY

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Objectives

Previous studies using the experience sampling method (ESM) have shown that affect plays a role in binge drinking (BD) [1]. However, no study using ESM has investigated the role of impulsivity and craving in BD.

Materials and methods

Female participants with a recent-onset (illness duration < 5 years) alcohol use disorder (AUD) and regular BD were included in an ongoing ESM study. On specific days over a 1-year period, participants were asked questions about impulsive behaviors, craving for alcohol as well as drinking behaviors. A mixed-effects polynomial regression model with linear, quadratic and cubic functions was used to model the separate pre- and post-binge trajectories of the within-person effects of impulsivity and craving. Only the ratings from 5 hours before or 5 hours after a BD episode were considered in the analyses.

Results

In total, 32 patients with AUD (mean age = 21.5 ± 3.4, mean BMI = 23.6 ± 2.6) reported 79 BD episodes with 127 antecedent and 124 consequent ratings. Both impulsivity and craving increased before a BD episode. Impulsivity increased curvilinearly with a significant linear ($p = 0.008$) and quadratic effect ($p = 0.014$) while craving increased curvilinearly with a significant cubic effect ($p = 0.025$). Afterwards, both impulsivity and craving decreased linearly ($p = 0.006$ and $p < 0.001$ respectively).

Conclusions

These preliminary results point to an increase in impulsivity and craving before a BD episode and a decrease afterwards. Interventions aimed at changing the course of impulsivity and craving before a potential BD episode could interesting in the treatment of AUD.

Keywords
Experience sampling method, craving, impulsivity, binge drinking

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NEUROINFLAMMATION STATUS IN RATS DURING PROLONGED ABSTINENCE AND AFTER ETHANOL REINTRODUCTION

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Objectives
Drug seeking and relapse are the main clinical problems related to Alcohol Use Disorders (AUDs). Accumulating evidence suggests that chronic alcohol consumption is associated with excessive oxidative damage and neuroinflammatory processes and these events have been associated to early alcohol withdrawal (Knapp et al., 2016). In the present research we wonder if brain neuroinflammation remains altered during prolonged withdrawal situations and whether this alteration can be correlated with relapse behavior in alcohol consumption.

Materials and methods
The effects of alcohol reintroduction were also evaluated. To this aim we have used a model based on the alcohol deprivation effect (ADE) within a cohort of wild-type male Wistar rats (Cano-Cebrián 2021). Two subpopulations were identified according to the alcohol relapse-like drinking behavior displayed (ADE and NO-ADE subpopulations). The levels of mRNA of different inflammatory mediators (IL-1B, IL6, TNFα, HMGB1, iNOS, NFκB and NLRP-3) in the prefrontal cortex of rats were quantified. All these analyses were performed in two different conditions: after 21-day alcohol deprivation (prolonged abstinence) and after 24 hours of ethanol reintroduction in both subpopulations.

Results
ADE and NO-ADE rats showed different endophenotypes. ADE rats always displayed a significant lower alcohol intake rate and ethanol preference than NO-ADE rats. The results also demonstrated the existence of altered brain neuroinflammation status after prolonged abstinence exclusively in ADE rats. Moreover, when ethanol was reintroduced in the ADE subpopulation, neuroinflammatory markers were restored.

Conclusions
All in all, our results suggest that brain neuroinflammation status could be key processes participating in the alcohol craving that will lead to the relapse process.

Keywords
alcohol relapse, alcohol deprivation effect, neuroinflammation

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ETHANOL INDUCES EXOSOME SECRETION VIA THE UPREGULATION OF MITOCHONDRIA-ASSOCIATED MEMBRANES

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Objectives

Extracellular vesicles (EVs) play an important role in the intercellular signaling in physiopathology. We previously showed that ethanol treatment increases the number of secreted EVs astroglial cells and their content in inflammatory molecules, spreading and amplifying the neuroinflammatory response. Considering that cholesterol/sphingomyelin are important for EVs biology, and the importance of mitochondria-associated endoplasmic reticulum membranes (MAMs) in their homeostasis, we have evaluated if MAMs and sphingomyelinases (SMases) could participate in ethanol-induced EVs release.

Materials and methods

We used BV2 microglial cell line and brains from C57/BL6 wild-type mice. EVs were isolated from the extracellular medium of BV2 cultures cells treated with or without ethanol at 10, 50 and 100 mM. Radioactive metabolic tracers and thin layer chromatography identification were used as a quantitative method to assay phospholipid transfer, sphingomyelinase activity and cholesterol uptake/esterification. Inhibitors of SMases (desipramine and GW4869) and MAMs (cyclosporin A) were also used to analyze the role of lipid metabolism in ethanol-induced alterations of EVs secretion.

Results

We demonstrated that ethanol increased EVs secretion and altered their content in BV2 microglial cells. Ethanol also activated MAMs, in a dose-dependent manner, in crude membrane fractions from murine brain and BV2 cells. Ethanol also alters the lipid metabolism by increasing cholesterol uptake and esterification, and SMase activity in BV2 microglia cells. Notably, the ethanol-induced increase in EVs secretion was abolished by using SMases or MAMs inhibitors in BV2 cells, which suggests the role of SMases and MAMs in ethanol-induced EVs secretion.

Conclusions

These results support the hypothesis that ethanol, by altering the lipid metabolism through the activation of both SMases and MAMs, increases the EVs secretion in microglial cells.

Keywords

Extracellular vesicles, lipid metabolism, mitochondria-associated ER membranes, alcohol, neuroinflammation, microglia, sphingomyelinases, phospholipids.

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SEX DIFFERENCES IN THE LIPIDOMIC PROFILE OF EXTRACELLULAR VESICLES OF ADOLESCENTS EXPOSED TO ALCOHOL INTOXICATION

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Objectives
Recent evidence shows the role of extracellular vesicles (EVs) as important cell communicators in pathological processes, such as inflammation. These vesicles contain lipids, which could participate in their biogenesis, release and invagination in the target cells. Considering the ability of EVs to cross the blood-brain barrier, being good non-invasive biomarkers, the aim of this study is to evaluate if alcohol consumption in adolescence could alter the lipidomic profile of plasma EVs and whether there are gender differences.

Materials and methods
To answer this question, we use plasma EVs from human adolescent males and females after an acute ethanol intoxication and their respective control individuals. By using a novel bioinformatic approach, we carried out the differential expression analysis and the biological functional characterization of the lipidomic data.

Results
The results show that there are significant differences in the lipidomic profile between plasma EVs from adolescent females and males, as well as there are also significant differences in the plasma EV lipid content from alcohol intoxicated and non-intoxicated individuals. Furthermore, the functional characterization of plasma EV lipidomic profile shows that some Fatty Acids and its corresponding super class Fatty Acyls, which are related to the inflammatory immune response, are upregulated in adolescent females after an alcohol intoxication, when compared with their non-alcohol female. These data support our previous results demonstrating that adolescent females are more vulnerable to the effects of alcohol (e.g., higher expression of proinflammatory molecules than males).

Conclusions
Therefore, we can conclude that the lipids of the plasma EVs could be good candidates as biomarkers of the neuroinflammation induced by alcohol consumption in adolescence and they can help us to explain the mechanisms underlying the inflammatory immune response.

Keywords
Extracellular vesicles, alcohol, lipidomics, differential expression, lipid set enrichment analysis, sex differences.

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EXPLORING THE ALCOHOL ANTI-RELAPSE EFFECT OF N-ACETYLCYSTEINE: STUDIES IN RATS

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Objectives

In previous research conducted in our laboratory, we evidenced the preclinical effectiveness of N-acetylcysteine (NAC) in the prevention of alcohol relapse (Cano-Cebrián et al., 2021). However, the mechanism underlying its anti-relapse efficacy is complex and still remains unclear.

Materials and methods

To explore it, a cohort of 30 male Wistar rats were used. 15 rats, individually housed, were continuously exposed to water (n=9) or alcohol (n=6) during 20 weeks. Additionally, another 15 rats were exposed to the Alcohol Deprivation Effect (ADE) model along 40 weeks. During the fifth abstinence period, rats were subcutaneously treated with vehicle or NAC (60 or 100 mg/kg; once per day; n=5/group) during ten days. All animals were euthanized and their brains processed.

Results

According to the literature (Berrríos-Campos et al, 2020), three potential mechanisms were explored: the expression of glutamate transporters (GLT-1, xCT and GLAST) in the nucleus Accumbens, the oxidative status (quantification of GSSG and GSH) in the hippocampus and also several neuroinflammation mediators were determined in the prefrontal cortex of the rats. In our experiments NAC did not have any effect on GLT1 nor xCT expression, and only NAC 60 mg/kg was able to rescue GLAST levels in nucleus Accumbens. On the other hand, our results clearly showed an impairment in oxidative balance in hippocampus during abstinence. Moreover, an increase in IL1β and TNFα expression was also detected. NAC (60 or 100 mg/kg) was able to blunt not only the prooxidant condition but also the increased inflammatory mediators determined under our experimental conditions.

Conclusions

All in all, our set of results suggest that the mechanism of action that underlies the alcohol anti-relapse effect of NAC is probably related to their antioxidant and anti-inflammatory properties.

Keywords

N-acetylcysteine, alcohol relapse, glutamate transporters, oxidative stress, neuroinflammation

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MOTOR STIMULANT EFFECTS OF DAMGO DIRECTLY INJECTED IN THE TAIL OF THE VTA OF RATS

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Objectives

Our previous research showed that the mu-opioid receptors (MORs) located in the GABA neurons of the posterior VTA are cruzial to explain the activating effects of ethanol on the VTA-DA neurons of the mesocorticolimbic system (Sánchez-Catalán et al., 2009). Ethanol exerts its activating effects on the VTA-DA neurons through the MORs-mediated inhibition of the VTA-GABA neurons. Consequently, direct injection of agonists of MORs such as DAMGO and Salsolinol (the ethanol-derived metabolite) in the posterior VTA are able to indirectly activate the VTA-DA neurons. The tail of the VTA (tVTA), also named the rostromedial tegmental nucleus (RMTg), is another midbrain structure that exerts a major inhibitory control on VTA-DA neurons (Kaufling et al., 2009). The tVTA is mostly composed of GABA neurons with a notable expression of MORs. The role displayed for this structure on the ethanol-induced activation of the VTA-DA neurons is not known. The aim of this study has been to initiate the analysis of the possible role displayed by the tVTA (and the MORs located in this structure) in the behavioural activation induced by ethanol. Here, we investigate the locomotor-activating effects of local microinjections of DAMGO within the tVTA of rats. We compare our present results with those previously obtained in the posterior VTA with this agonist.

Materials and methods

For this purpose, a cohort of Sprague Dawley rats were divided into four groups that received aCSF or DAMGO (0,13 nmol) intra-tVTA after a local pre-treatment (36-48 hours before) with aCSF or β-FNA (2,5 nmol), an antagonist of μ-opioid receptors. Later, locomotor activity was registered for 30 minutes.

Results

The local administration of DAMGO within the tVTA caused an increase in locomotor activity up to 300% above baseline, an increase very similar to that observed in our previous studies in the posterior VTA. The intra-tVTA pretreatment with β-FNA prevented this increase, suggesting that tVTA-GABA neurons and the MORs expressed in these neurons could also participate in the behavioural effects of ethanol.

Conclusions

Future experiments will be aimed at evaluating whether intra-tVTA administration of ethanol could be also capable of indirectly activate the DA mesocorticolimbic system through MORs.

Keywords

tVTA, RMTg, Locomotor activity, Opioid receptors, Ethanol, rats

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ESTIMATION OF SALVE FIBROSIS STAGE BY VIBRATION-CONTROLLED TRANSIENT ELASTOGRAPHY (VCTE) IN COMPENSATED AND DECOMPENSATED ALCOHOL-RELATED LIVER DISEASE (ALD).

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Objectives
Non-invasive staging of fibrosis is an important tool to predict prognosis in alcohol-related liver disease (ALD). Decompensation of ALD is mostly due to alcoholic hepatitis which may be associated with cholestasis. Inflammation and cholestasis have been shown to increase liver stiffness (LS) and thus could bias non-invasive assessment of fibrosis in particular in patients with decompensated ALD. The aim of our study was to compare the utility of VCTE measurement for non-invasive staging in patients with compensated and decompensated ALD.

Materials and methods
We analyzed a multinational cohort of ALD patients who underwent percutaneous or transjugular liver biopsy and vibration-controlled transient elastography (VCTE) using FibroScan 502 Touch. Histological fibrosis was classified using the recently developed ALD-specific SALVE staging system into no fibrosis (SALVE fibrosis stage, SFS 0), mild fibrosis (SFS 1-2), severe fibrosis (SFS 3), cirrhosis (SFS 4A/AP-4B/BP) and severe cirrhosis (SFS 4C/CP) (1). Alcohol-related steatohepatitis (ASH) was defined as presence of hepatocellular ballooning plus neutrophil infiltration. Presence of canalicular and ductular cholestasis was assessed.

Results
The cohort included 179 patients with ALD (compensated, 106; decompensated, 73). Compared with compensated ALD, decompensated ALD showed higher frequency of ASH (68% vs. 12%) and cholestasis (61% vs. 9%). Median LS was significantly higher in decompensated than in compensated ALD both overall (75.0 vs. 10.2 kPa, p

Conclusions
LS values obtained by VCTE are higher in decompensated vs. compensated ALD irrespective of histological stage. In decompensated ALD, VCTE using shear wave frequency of 50 Hz is not useful to assess distinct prognostic stages due to ceiling effects for LS.

Keywords
alcohol-related liver disease, fibrosis, cirrhosis, noninvasive, transient elastography

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ASSESSING ATTENTIONAL BIAS THROUGH THE ALCOHOL HAYLING TASK IN YOUNG COLLEGE STUDENTS WITH AND WITHOUT A BINGE DRINKING PATTERN

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Objectives

Craving and attentional bias towards alcohol-related stimuli play a central role in the development of addiction (Addolorato, Leggio, Abenavoli, Gasbarrini & Alcoholism Treatment Study Group, 2005; Drobes & Thomas, 1999; Cox, Hogan, Kristian & Race, 2002; Fadardi & Cox, 2009; Naqvi et al., 2015). The craving dimensions involved, such as expectations or compulsivity, are of notable importance from a preventive and interventional point of view (Schneekloth et al., 2012). However, the relationship between alcohol craving/consumption and alcohol attentional bias remain poorly understood, especially when considering craving dimensions in young people who binge drink - the most common pattern of alcohol misuse during late adolescence.

Materials and methods

Seventy-five college students from the University of Minho performed an adapted version of the Alcohol Hayling task (Rose, Mason-Li, Nicholas & Hobbs, 2010) – an alcohol-related sentence-completion tool which measures alcohol attentional bias (AAB) and inhibitory control (IC). Additionally, alcohol consumption and craving were measured through the Alcohol Use Disorder Identification Test (AUDIT) and the Alcohol Craving Questionnaire – Short Form – Revised (ACQ-SF-R), respectively. Multiple regression models were used in order to evaluate potential relationships between AAB/IC and alcohol consumption and craving.

Results

Higher craving from compulsivity and purposefulness dimensions of the ACQ-SF-R were associated with AAB measured by number of alcoholic words and latency in the alcoholic part of the Alcohol Hayling task. Levels of alcohol consumption did not show an association with AAB or IC variables.

Conclusions

AAB in young alcohol users was specifically related to compulsivity and purpose dimension of craving. This study suggests that specific craving intensity dimensions could be a stronger determinant of attentional bias than the level of alcohol consumption in young alcohol users. These findings are of special importance for more targeted prevention strategies in at risk population before an addiction develops.

Keywords

alcohol consumption, alcohol craving, compulsivity, purposefulness, attentional bias, inhibitory control

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IMPACT OF COVID-19 PANDEMIC ON HOSPITAL ADMISSIONS FOR ALCOHOL SPECIFIC CONDITIONS.

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Objectives

To describe the effect of the Covid-19 pandemic on hospital admissions for wholly alcohol attributable conditions.

Materials and methods

This retrospective cohort study was conducted at Nottingham University Hospitals (NUH), United Kingdom. Cohorts were defined as pre-pandemic (March-2019 to February-2020) and pandemic (March-2020 to February-2021). Conditions were defined as alcohol specific where alcohol was the sole cause and their alcohol attributable fraction was 1.0 (100 per cent), as per Public Health England (2014 and 2020) guidance. All adult patients (age >16years) admitted to NUH with alcohol specific primary diagnoses during the two defined study periods were included. Descriptive comparisons were made between the groups with respect to alcohol specific primary diagnosis, liver-specific primary diagnosis, gender, age, ethnicity, civil status, length of stay and socioeconomic background.

Results

A total of 255,824 patients were admitted to NUH during the two study periods (pre-pandemic n=131,022 and pandemic n=124802). Of them 1484 patients had an alcohol specific primary diagnosis and were included in final analysis. Pandemic, compared to pre-pandemic, had a significantly higher number of alcohol specific admissions (811 versus 673, p

Conclusions

Although during the Covid-19 pandemic total number of hospital admissions reduced a significantly higher number of patients were admitted due to an alcohol specific condition and had underlying liver disease. Patients admitted during the pandemic had a longer length of stay and observed extreme variation in socioeconomic background.

Keywords

Alcohol Alcohol related liver diseases Retrospective Alcohol specific conditions

References

Please refer to table 1 included in the attached word document.

HISTOLOGICAL LESIONS CAN PREDICT RESPONSE TO CORTICOSTEROIDS IN PATIENTS WITH SEVERE ALCOHOL-RELATED STEATOHEPATITIS (ASH).

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Objectives

Liver biopsy is useful to confirm alcohol-related steatohepatitis (ASH) in patients with alcohol-related liver disease (ALD) and clinically suspected alcoholic hepatitis (AH). Current EASL guidelines recommend prednisolone for the treatment of severe AH with Maddrey´s discriminant function (MDF) ≥32 and assessment of treatment response at day 7 using the Lille model. The aim of our study was to investigate the potential utility of histologic features of ALD for early prediction of response to corticosteroids as per Lille score in patients with AH in order to avoid the risk of a 7-day steroid exposure for those unlikely to benefit.

Materials and methods

We analyzed data of a multinational cohort of patients with severe AH and MDF ≥32. All patients underwent liver biopsy for the confirmation of clinically suspected AH and were treated with prednisolone. Morphological features of ALD including steatosis, activity, canalicular and ductular cholestasis as well as fibrosis stage were assessed using the recently developed ALD-specific SALVE grading and staging system (1). Association of histological variables with response to steroids (Lille score

Results

Complete data were available in 119 patients. A Lille score of

Conclusions

Liver biopsy may thus be used not only to confirm ASH but also to achieve early prediction of steroid efficacy thus sparing high-risk AH patients from potentially life-threatening side effects of steroid exposure.

Keywords

alcohol-related liver disease, steatosis, fibrosis, cholestasis, prednisolone

References

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DEVELOPMENTAL ALCOHOL EXPOSURE STUNTS THE MATURATION OF MULTISENSORY INTEGRATION

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Objectives
Children with FASD often present sensory alterations such sensory overload, attention deficits, poor visual-motor integration, delayed auditory processing and hypersensitivity to tactile stimulation. There is growing evidence that these sensory problems give rise to social problems and learning deficits. We work with the overarching hypothesis that developmental alcohol exposure disrupts the activity-dependent neuronal plasticity that is needed for circuit refinement in sensory cortices, resulting in abnormal multisensory integration (MSI). Here we tested the prediction that exposure to alcohol between postnatal (P) days 10 to 30 (roughly equivalent to the last months of human gestation) would lead to a persistent disruption or MSI in the ferret rostral posterior parietal cortex (PPr).

Materials and methods
Ferrets were exposed to alcohol (25% in saline, 3.5g/Kg, i.p.) between P10-P30. After this period of alcohol exposure we conducted in vivo electrophysiology recordings at P70-P100 (infancy group) or P180-P200 (adolescence group). Neuronal responses in PPr were recorded after visual only (V), tactile only (T) or combined visual-tactile (VT) stimulation. Neurons that respond to both V and T stimulation are categorized as bimodal. These bimodal neurons can be further categorized by displaying MS facilitation (When VT is higher than V and T), MS depression (When VT responses are smaller than V and T) or non- integrative (VT is indistinguishable from V and T). We have conducted a second experiment in normal animal comparing rates of multisensory integration during early, mid and late adolescence.

Results
We observed an effect of age and alcohol exposure on MSI. Accordingly, our findings show that during development there is a significant reduction and increase in multisensory depression and facilitation respectively. Therefore, rates of facilitation were higher in the adolescent saline group followed by adolescent alcohol, infant saline, and alcohol saline. The opposite order was observed for rates of depression. Our findings showing that MSI is different between infancy and adolescence prompted us to test in normal animals whether MSI continues to mature through the adolescence. Accordingly, we observed a progressive increase in facilitation and decrease in depression from early, mid to late adolescence.

Conclusions
Our findings show that alcohol exposure during the third trimester equivalent of human gestation stunts the maturation of MSI in ferret PPr. These findings help us to understand commons sensory deficits observed in FASD. In addition, we also shown in normal animals that MSI continues to mature throughout the adolescence.

Keywords
Fetal Alcohol Syndrome Visual system Somatosensory system Sensory processing

References

FIMBRIA/FORNIX MICROSTRUCTURE IS VULNERABLE TO ALCOHOL CONSUMPTION AND AFFECTS HIPPOCAMPAL-PREFRONTAL CORTEX CONNECTIVITY DURING ABSTINENCE

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Objectives

Alcohol dependence is characterized by a gradual reduction in cognitive control and inflexibility to contingency changes. However, the neuroadaptations underlying this aberrant behavior, especially of those associated to relapse during abstinence, are poorly understood. Recently, we reported microstructure alterations in white matter (WM) of both, alcohol use disorder (AUD) patients and excessively drinking rats (De Santis et al. 2019), that progressed during early abstinence. Here in a new translational study we report on the neurobiological consequences of these alcohol-induced WM alterations in alcohol-dependent patients and rats.

Materials and methods

Effect size of fractional anisotropy (FA) reduction in AUD patients (N=48) vs. age matched healthy controls (n=36) in different WM tracts was calculated from diffusion tensor imaging (DTI). Fimbria/fornix tract damage in patients was correlated with cognitive test performance inthe Number-Symbol Test (NST), and Trail-Making Tests A and B (TMT-A, TMT-B). A total of 48 rats that underwent chronic intermittent exposure to alcohol, i.e. post-dependent (PD) rats were used for WM microstructure assessment with DTI and histology, and functional analysis with electrophysiological techniques.

Results

The largest FA reduction in AUD patients was found in the fimbria/fornix tract (fi/fo). This effect showed a significant and positive correlation with NST and negative correlations with TMT-A and TMT-B, establishing an association in AUD patients between fi/fo microstructure and reduced cognitive flexibility and information processing capacity. In PD rats, DTI analysis unveiled microstructural alterations in the fi/fo consistent with demyelination that was confirmed histologically. Finally, electrophysiological recordings in the hippocampus (HC) and the prefrontal cortex (PFC), two fi/fo interconnected structures, demonstrated reduced effective connectivity.

Conclusions

Our results unveil a high microstructural vulnerability of the fi/fo tract to alcohol exposure that compromises HC-PFC communication during abstinence and could explain important cognitive deficits found in AUD patients, such as reduced cognitive flexibility.

Keywords

Patients, post-dependent rats, fimbria/fornix, hippocampus, prefrontal cortex, DTI, MRI, electrophysiology, myelin, memory, cognitive flexibility

References

De Santis et al. Microstructural white matter alterations in men with alcohol use disorder and rats with excessive alcohol consumption during early abstinence. JAMA Psychiatry. 2019;76(7):749-758.

KNOWLEDGE AND PRACTICE TOWARDS ALCOHOL CONSUMPTION IN A SAMPLE OF UNIVERSITY STUDENTS

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Objectives
Alcohol affects many human systems and is involved in the pathogenesis of other diseases. Particular attention must be paid to alcohol consumption among young people. It has been shown that 25% of young people's deaths are attributable to alcohol, and around 35 million people aged over 11 had consumed at least one alcoholic beverage in 2015

Materials and methods
Young people aged 18–24 were the most vulnerable to binge drinking in Italy, and 50.6% of teenagers drunk alcohol. Only a few studies in the literature have investigated those habits in university students. This study aims to examine alcohol use habits in a population of university students in Italy. Between 2018 and 2019, an anonymous online questionnaire was randomly sent to university students from 17 different universities in a network of research centres to study alcohol use disorders. The survey included socio-demographic information, questions about alcohol use, knowledge about alcohol consumption, and related risks. Used questionnaires were the Alcohol Use Disorders Identification Test-Consumption (AUDIT-C) and the Drinking Motive Questionnaire-Revised (DMQ-R).

Results
Results: the AUDIT-C revealed that 53.3% of students were high-risk drinkers. Regarding binge drinking habits, 13.1% of students admitted to binge drinking behavior at least once a month. In our sample, male students are more likely to be low-risk drinkers than female peers ($p < 0.008$). Students from northern Italy are more likely to be high-risk drinkers ($p=0.003$). Beer (65.9%) and wine (60.9%) were the most consumed alcoholic beverages. The most common places to drink alcohol were pubs (85.5%). The most likely motivations to drink alcohol were enhancement (40.43%), social (38.39%), coping (15.63%), and social pressure or conformity (5.55%). Only 43.8% of participants reported having attended an educational course on alcohol.

Conclusions
University students were not fully aware of the implications of alcohol misuse and will be part of the adult society as critical figures and future leaders. It is imperative to inform students about alcohol consumption risks and investigate the motivations to drink. Stress, anxiety, and social pressure are only a few issues young people are exposed to. Special attention must be paid to young people and their coping strategies that involve substance abuse by using educative, preventive, and motivational approaches.

Keywords
FASD; abuse; alcohol; student; university; medical faculty

References

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MARKERS OF NEUROINFLAMMATION IN THE SERUM OF PREPUBERTAL CHILDREN WITH FETAL ALCOHOL SPECTRUM DISORDERS

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Objectives

Fetal alcohol spectrum disorders (FASD) are the manifestation of the damage caused by alcohol consumption during pregnancy. Children with Fetal Alcohol Syndrome (FAS), the extreme FASD manifestation, show both facial dysmorphology and mental retardation. Alcohol consumed during gestational age prejudices brain development by reducing, among others, the synthesis and release of neurotrophic factors and neuroinflammatory markers. Alcohol drinking induces also oxidative stress. The present study aims at investigating the potential association between neurotrophins, neuroinflammation and oxidative stress in 12 prepubertal male and female FASD children diagnosed as FAS or partial FAS (pFAS).

Materials and methods

Accordingly, we analyzed, in the serum, the level of BDNF and NGF and the oxidative stress, as free oxygen radicals test (FORT) and free oxygen radicals defense (FORD). Moreover, serum levels of inflammatory mediators (IL-1α, IL-2, IL-6, IL-10, IL-12, MCP-1, TGF-β and TNF-α) involved in neuroinflammatory and oxidative processes have been investigated.

Results

We demonstrated in pre-pubertal FASD children low serum levels of NGF and BDNF, respect to healthy controls. These changes were associated with higher serum presence of TNF-α and IL-1α. Quite interestingly, an elevation in the FORD was also found despite normal FORT levels. Moreover, we found a potentiation of IL-1α, IL-2, IL-10 and IL-1α1 in the analyzed female compared to male children.

Conclusions

The present investigation shows an imbalance in the peripheral neuroimmune pathways that could be used in children as early biomarkers of the deficits observed in FASD.

Keywords

Cytokine; Inflammation; ROS; NGF; BDNF; Child.

References

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EFFECTS OF ALCOHOL HANGOVER ON ATTENTIONAL BIAS TOWARDS ALCOHOL-RELATED STIMULI - AN ELECTROENCEPHALOGRAPHY STUDY

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Objectives
Binge drinking (BD), an excessive but episodic alcohol use pattern, often associated with adolescence and youth, has been become a major public health problem due to the special vulnerability of this population to the neurotoxic effects of alcohol. Alcohol attentional bias has been described as a relevant marker in alcohol misuse (Field & Cox, 2008) and recent research has shown that alcohol hangover –i.e., aversive symptoms, which follow the excessive drinking– may affect alcohol cue reactivity (Gunn et al., 2021). Nevertheless, to the best of our knowledge, brain activity underlying alcohol cue processing in young binge drinkers during alcohol hangover remains unexplored.

Materials and methods
Electroencephalographic (EEG) activity was recorded from a total of 11 BD college students (6 females; 5 males), in two moments: a regular day – without BD in the 72h prior-, and a day following a BD event, while participants experienced alcohol hangover. On both days, participants performed a visual novelty oddball task, with alcohol-related stimuli as distractors. The P3 event-related potential (ERP) was obtained and analyzed. Craving for alcohol was assessed before both EEG collections.

Results
ERP analysis of EEG signal during alcohol hangover revealed that binge drinkers showed a significantly augmented P3 amplitude towards alcoholic cues when compared to a normal day. Contrarily, no differences were shown regarding P3 amplitude towards the target stimuli. Additionally, participants reported higher levels of alcohol craving during hangover.

Conclusions
Our findings are congruent with recent literature suggesting an augmented attentional bias towards alcohol-related stimuli in young binge drinkers, associated with increased craving (Field & Eastwood, 2005; Weafer & Fillmore, 2013). These results may have valuable implications, as this increased P3 amplitude during hangover could contribute to the upkeep of the consumption pattern, which, as explained through the continuum hypothesis may develop into the early stages of an alcohol use disorder (Almeida-Antunes et al., 2021).

Keywords
Attentional Bias; Alcohol Hangover; Binge Drinking; Electroencephalography

References

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LIVER IRON OVERLOAD IN ALCOHOLIC LIVER DISEASE: CROSSTALK BETWEEN ENDOTHELIAL CELLS AND HEPATOCYTES IN IRON REGULATION

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Objectives

Cancerogenic hepatic iron accumulates in patients with alcoholic liver disease, but the exact mechanism, namely the role of the systemic master switch hepcidin is still poorly understood. In addition, bone morphogenetic protein 6 (BMP6) derived from liver sinusoidal endothelial cells (LSECs) and the BMP6/SMAD signaling pathway is considered to be essential for hepcidin expression. Although LSEC-specific deletion of BMP6 causes hepcidin deficiency and iron overload in mice, the direct iron sensing of ECs is still incompletely understood. We here study directly the crosstalk between endothelial cells (ECs) and hepatocytes iron metabolism.

Materials and methods

Hepatocytes (HC) (Huh7, primary mouse hepatocytes) were direct co-cultured with ECs, including HUVECs and SK hep, and ECs conditioned media were also used to culture hepatocytes. To explore the reactions ECs to surrounding iron they were exposed to different concentrations of ferric ammonium citrate and hemin. ECs were also exposed under EtOH and the ECs conditioned medium was collected for hepatocytes culturing.

Results

Direct coculture with ECs or EC-conditioned media both significantly increased hepatocellular hepcidin and its upstream regulators pSMAD1/5/8 and SMAD1. Blockage of hepcidin upregulation by an ALK inhibitor, BMP6 blocking antibody or BMP6 siRNA, identified EC-secreted BMP6 as major pathway for controlling hepcidin in crosstalk. In addition, EC-derived BMP6 in the coculture system responded highly sensitive to levels of iron as low as 0.5 µM within 6 hours. EtOH in the ECs conditioned medium also induced BMP6/hepcidin expression.

Conclusions

We here establish a hepatocyte-endothelial coculture system to recapitulate iron sensing by hepcidin through EC-derived BMP6. Furthermore, ECs are highly sensitive and responsive to surrounding iron or ethanol. Our coculture system should provide a useful tool to further elucidate alcohol-mediated disturbances in iron homeostasis.

Keywords

Endothelial cells; BMP6/SMAD pathway; Hepcidin; Iron; Ethanol

References

None

ALCOHOL-RELATED LIVER DISEASE, HEMOLYSIS AND IRON SIGNALING

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Objectives

The liver is the main target organ of heavy alcohol consumption eventually causing alcohol-related liver disease (ALD), but our recent study indicates that red blood cells (RBC) are also an important target in alcoholic injury, and the dysfunctional liver cells can aggravate.

Materials and methods

Based on a large prospectively enrolled patient cohort of heavy drinkers (Heidelberg cohort), we identify a subgroup of 25% with high ferritin and low hemoglobin levels that show the worst outcome. Mice models of mild and severe hemolysis were built by once or double phenylhydrazine (PHZ) injection. In vitro, we used endothelial cells and hepatocytes co-culture to observe heme effects on hepcidin regulation.

Results

In the subgroup of patients with low hemoglobin and high ferritin, the large erythrocytes (high MCV), elevated unconjugated bilirubin and LDH before or after the detoxification are highly suggestive of enhanced hemolysis. This is further confirmed by the high expression of CD163, a heme scavenger. Surprisingly, although the challenge with the hemolytic agent phenylhydrazine (PHZ) demonstrated elevated RBC fragility in ALD patients, levels of folic acid and B12 were comparable to controls. Moreover, despite iron overload in these patients, serum levels of the iron master switch hepcidin were suppressed ultimately causing further iron uptake. In mouse models, mild hemolysis induced hepcidin while severe hemolysis causes a paradox downregulation. Finally, in vitro heme strongly upregulates endothelial cell-derived BMP6, the most important signaling molecule upstream of hepcidin. In contrast, iron release from severe heme degradation causes a direct blockage of hepatocellular BMP/SMAD signaling pathway.

Conclusions

Our translational findings suggest a novel role of heme turnover in hemolytic damage of patients with ALD and systemic iron overload due to altered hepatocellular hepcidin signaling, which then forms a vicious circle between blood and liver.

Keywords

Liver, hemolysis, alcohol, iron

References

No

THE ROLE OF PNPLA3, MBOAT7 AND TM6SF2 DURING ALCOHOL DETOXIFICATION: DIFFERENT MECHANISMS OF FIBROSIS AND STEATOSIS DEVELOPMENT

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Objectives

In genome wide association studies PNPLA3, MBOAT7 and TM6SF2 were identified as important risk genes for the development of alcoholic cirrhosis, however, their functions and molecular mechanisms are still poorly understood. We here present first data on the role of PNPLA3, MBAOT7 and TM6SF2 genotypes on liver stiffness (LS), steatosis (CAP) and inflammation during alcohol withdrawal.

Materials and methods

763 patients with ALD which were hospitalized for alcohol withdrawal at Salem Medical Center between 2007 and 2018 were genotyped for PNPLA3 s738409, MBOAT7 rs626283 and TM6SF2 rs58542926 polymorphisms. All patients had routine laboratory, abdominal ultrasound and a transient elastography measurement (FibroScan) at admission. In 512 patients, data after 6.3 days of alcohol withdrawal was available.

Results

71% of the patients were male, median age was 52 years, median BMI was 24.7 kg/m2 and median alcohol consumption was 163 g/day. At admission, no difference between the genotypes of PNPLA3, MBOAT7 and TM6SF2 was seen regarding age, BMI, gender, alcohol consumption or transaminase levels. Significant differences were observed for PNPLA3 and MBOAT7 during alcohol detoxification. While MBOAT7 was associated with higher LS, no differences were observed between genotypes upon alcohol detoxification. In contrast, PNPLA3 caused clearly a delayed resolution of LS during withdrawal of alcohol due to inflammation. This could be recapitulated when looking at serum markers of liver inflammation. TM6SF2 showed no effect on alcohol withdrawal regarding LS, CAP or transaminases. In a sub-analysis of n=108 liver biopsies, inflammation was highly associated with PNPLA3 but not MBOAT7 and TM6SF2. More interestingly, PNPLA3 was associated with higher steatosis (CAP). A multivariate model confirmed that PNPLA3 was associated with steatosis and inflammation but not fibrosis. MBOAT7 was only associated with fibrosis/cirrhosis but not inflammation or steatosis.

Conclusions

These first genotype data on a “human alcohol knock-out” intervention underscore important difference between the three genes. PNPLA3 seems to primarily drive fibrosis through inflammation while MBOAT7 seems to have a direct effect on fibrosis. TM6SF2 showed no effect at all. Finally, alcohol detoxification could be a novel interventional approach to further dissect metabolic mechanisms and their associations with genotypes.

Keywords

Liver stiffness, ALD, PNPLA3, MBOAT7

References

no references

SURVIVAL IN A 10 YEAR PROSPECTIVE COHORT OF HEAVY DRINKERS: LIVER STIFFNESS IS THE BEST LONG-TERM PROGNOSTIC PARAMETER

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Objectives
Alcoholic liver disease (ALD) is the most common liver disease in the western world. Although measurement of liver stiffness (LS) by transient elastography has been well established for early diagnosis of fibrosis, no prospective long-term data on survival exist so far in patients with ALD. We here present first data on the prognostic impact of LS on long-term survival of Caucasian heavy drinkers in a 10 year, prospective single center trial.

Materials and methods
Information of survival status was obtained in 675 (71.6%) of 943 screened patients that had presented for alcohol detoxification (6.0 days) over a 10-year period from 2007-2017 with a mean daily consumption of alcohol of 178 g. Mean observation time was 3.7 years and mean duration of heavy drinking was 14.0 years. All patients had LS measurements by transient elastography and routine laboratory tests.

Results
During the observation time, 106 patients (15.7%) died. The cause of death could be clarified in 42 patients (39%) and it was liver-related in 16 (38%). Overall death was highest associated with LS (r=0.291, P=1.3E-14), followed by hemoglobin and alkaline phosphatase (AP). In a multivariate proportional hazard model, LS next to age, AP and serum albumin was the most significant independent predictor of survival with a hazard ratio of 1.013 (1.003 to 1.023, P

Conclusions
We here identify LS as the best long-term prognostic parameter in patients who heavily consume alcohol. LS measurements should become an important parameter for the screening of alcoholics.

Keywords
ALD, Liver stiffness, Survival

References
No references

H_2O_2-MEDIATED AUTOPHAGY DURING ETHANOL METABOLISM

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Objectives

Alcoholic liver disease (ALD) is the most common liver disease worldwide and its underlying molecular mechanisms are still poorly understood. Moreover, conflicting data have been reported on potentially protective autophagy, the exact role of ethanol-metabolizing enzymes and ROS.

Materials and methods

Expression of LC3B, CYP2E1, and NOX4 was studied in a mouse model of acute ethanol exposure by immunoblotting and immunohistochemistry. Autophagy was further studied in primary mouse hepatocytes and huh7 cells in response to ethanol and its major intermediator acetaldehyde. Experiments were carried out in cells overexpressing CYP2E1 and knock down of NOX4 using siRNA. The response to external H2O2 was studied by using the GOX/CAT system. Autophagic flux was monitored using the mRFP-GFP-LC3 plasmid, while rapamycin and chloroquine served as positive and negative controls

Results

Acute ethanol exposure of mice over 24 hours significantly induced autophagy as measured by LC3B expression but also induced the ROS-generating CYP2E1 and NOX4 enzymes. Notably, ethanol but not its downstream metabolite acetaldehyde induced autophagy in primary mouse hepatocytes. In contrast, autophagy could only be induced in huh7 cells in the presence of overexpressed CYP2E1. In addition, overexpression of NOX4 also significantly increased autophagy, which could be blocked by siRNA mediated knock down. The antioxidant N-acetylcysteine (NAC) also efficiently blocked CYP2E1- and NOX4-mediated induction of autophagy. Finally, specific and non-toxic production of H2O2 by the GOX/CAT system as evidenced by elevated peroxiredoxin (Prx-2) also induced LC3B which was efficiently blocked by NAC. H2O2 strongly increased the autophagic flux as measured by mRFP-GFP-LC3 plasmid

Conclusions

We here provide evidence that short-term ethanol exposure induces autophagy in hepatocytes both in vivo and in vitro through the generation of ROS. These data suggest that suppression of autophagy by ethanol is most likely due to longer alcohol exposure during chronic alcohol consumption with the accumulation of e.g. misfolded proteins.

Keywords

Alcohol liver disease (ALD); Ethanol metabolism, Cytochrome P450 2E1(CYP2E1), NADPH oxidase (NOX), Hydrogen Peroxide (H2O2), Reactive Oxygen Species (ROS).

References

None

ERYTHROPHAGOCYTOSIS BY MACROPHAGES TRIGGERS HO-1 AND HEPCIDIN REGULATION

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Objectives

So far, hepatic iron overload and hepcidin regulation in alcoholic liver disease (ALD) patients are still poorly understood. 200 billion red blood cells (RBCs) are produced every day, containing 6 g of hemoglobin and 20 mg of iron. The making and breaking of RBCs are at the heart of iron physiology. Hemolysis and fragile RBCs are easily observed in ALD patients, which highly lead to the iron (e.g., heme) releasing from the damaged RBCs or/and erythrophagocytosis conducted by macrophages. Mild but chronic iron releasing from the damaged RBCs triggered by long-term alcohol drinking could be an important reason for iron overload in ALD patients. However, how iron released from damaged RBCs influences hepcidin regulation and triggers hepatic iron deposition in ALD still keep unclear. Here, we are trying to mimic the crosstalk between RBCs and macrophages (THP1 cell line), and uncover the mechanisms of erythrophagocytosis on iron metabolism, namely hepcidin regulation, for providing more evidence to understand erythrophagocytosis, iron recycling, and iron overload in ALD patients.

Materials and methods

Erythrocytes took from the health donator were incubated with CuSO4 for oxidizing 2 hours, following with co-incubation with pre-differentiated THP-1 cells. Various %Htc and time points were performed. Heme oxygenase-1 (HO-1), hepcidin, and CD163 were detected as the iron sensing and regulation targets by q-PCR or/and western blotting. Hemin and water lysed red blood cells (RBCs) were directly incubated with THP-1 cells for mimicking the heme released from the hemoglobin. Finally, haptoglobin and hemopexin (Hpx) were added to form the complex of Hb/Hp or Heme/Hpx for replicating the physiological condition in the body.

Results

24 hours 0.01% to 1% hematocrit (Htc) incubation significantly induced heme HO-1 mRNA and protein expression in THP-1 cells. Hepcidin mRNA and CD163 were parallelly induced by Htc in a dose-dependent manner. In addition, 1% oxidized red blood cells (RBCs) induced HO-1 level in THP-1 cells in a time-dependent manner (from 2 to 24 hours). Hepcidin mRNA and CD163 were up-regulated in line with HO-1 induction. Furthermore, THP-1 cells were incubated with different dosages of hemin (from 1.25 to 30 μM) for 24 hours. HO-1 was gradually induced. However, the induced HO-1 and hepcidin mRNA levels by hemin were blunted by haptoglobin or hemopexin co-treatment.

Conclusions

We here established erythrophagocytosis model with CuSO4 oxidized RBCs and THP-1 co-incubation provides preliminary evidence that heme released from damaged RBCs interacts with macrophages and further triggered iron disturbance. And the CD163-HO-1-hepcidin pathway is highly involved in this regulation. Our data shed new light for better understanding the underlying mechanisms of iron metabolism-related diseases, for instance, iron deposition in alcoholic liver disease.

Keywords

Alcohol liver disease (ALD), Erythrophagocytosis, Red blood cells, Macrophages, Heme oxygenase-1 (HO-1), hepcidin.

References

None

REVISITING HEMODYNAMIC PROGNOSIS IN SEVERE ALCOHOLIC HEPATITIS

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Background and Aims: Severe alcoholic hepatitis (SAH) bears high rates of mortality. There are several predictors of mortality: serologic markers such as the Maddrey discriminant function (DF) and the MELD score, and hepatic venous pressure gradient (HVPG) measurement. HVPG was validated as the best surrogate marker for portal hypertension, and was associated with esophageal varices, development of ascites, risk of liver cancer and also in-hospital mortality in SAH. In this respect, the cutoff value ≥ 22 mmHg was considered a marker of bad prognosis. However, there is only one study - dating back in 2007, evaluating the latter correlation and the study population was rather limited. Therefore, we aimed to investigate the role of HVPG in predicting short and medium term mortality in SAH patients.

Method: Consecutive patients with a clinical suspicion of SAH, submitted for transjugular liver biopsy for diagnostic reasons were enrolled. All patients underwent HVPG measurement (7Fr balloon catheter) alongside liver biopsy. Baseline liver function tests were also recorded. 30 and 90 days mortality were recorded during follow-up.

Results: 103 biopsy proven HAS patients (74% males, median age 53 [32-74] years) were included. 11 (10.7%) patients died at 28 days and 8 more (18.4%) deceased at 3 months; 6 additional patients were lost from the follow-up. 75(72.8%) patients presented with ascites, 20(19.4%) with variceal bleeding and 22(21.4%) with encephalopathy. 38(36.9%) patients were infected at admission, of which 3(2.9%) had sepsis; 29 additional patients developed infection 48h after admission. In the end, 74(71.8%) patients received Prednisone, out of which only 54 also finalized the therapy.
HVPG showed a moderate ability to predict 28 days mortality (AUROC=0.721[95%CI:0.461-0.982]), not different from DF (AUROC=0.765[0.606-0.925]) and MELD (AUROC=0.700[0.533-0.866]).
The best cut-off value was 23.5 mmHg, which showed a good correlation with mortality (Fischer exact test p (F) = 0.003), a sensitivity (Se) of 0.97, specificity (Sp) of 0.25, positive and negative predictive values (PPV, NPV) of 79% and 75%, respectively; it correctly classified 73/93(78.5%) of patients.
The previously validated cut-off value for HVPG (22mmHg) correlated mildly with 28-days mortality (F=0.06), showing 0.75 Se and 96% NPV and correctly classifying 57/93 (61.3%) patients.
26.5 mmHg and 12 mmHg thresholds showed very good performances in predicting 28-days mortality (93%PPV) and 28-days survival (100%NPV), respectively.
DF and MELD were also independent predictors of mortality (OR of 1.12 and 1.29, respectively); the OR for HVPG was 1.65.
HVPG ≥23.5 mmHg also predicted 90-days mortality (F=0.01), while 22 mmHg cut-off did not.

Conclusion: HVPG is an adequate predictor of short-term survival, not inferior to DF and MELD. The best cut-off value (23.5 mmHg) also predicts 90-days mortality. HVPG<12 mmHg accurately predicts 28-days survival.

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