EX527 induces an increase in acetylation of H3K9 but decrease overall methylation.

Conclusions: The results of our work indicate for the first time that PFD can regulate epigenetic marks possibly through modulation of the PPARγ-SIRT1-DNMT1 axis. Acetylation in H3K9 decreases with PFD treatment, however overall methylation increases. The perspectives of this work will be to analyze the methylation of specific genes (PPARalpha, IL-6, TNFalpha) involved in the development of liver diseases.

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14

Molecular, histological and biochemical changes in a NASH murine model whit a diet high in fats and sugars

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Background and aim: The increase in NASH prevalence coincides with the current obesity pandemic. Obesity is characterized by a state of chronic inflammation with oxidative stress in adipose tissue and liver. A high fat/sugar diet can induce non-alcoholic steatohepatitis, which is characterized by inflammation, hepatocyte swelling, and steatosis. To assess molecular, histological, and biochemical changes in a murine NASH model subjected to a high-fat diet for 16 weeks.

Material and methods: Male mice 4-5 weeks old, C57BL / 6J were fed a high-fat diet (HF, 60% fat, 42gr / L sugars in water) for 16 weeks. Every 4 weeks 4 mice were sacrificed for a follow-up of the model at 4, 8, 12 and 16 weeks. Serum glucose was measured after 4 hours of fasting, animal weight and caloric intake. The liver was removed and weighed, as was the epididymal adipose tissue. AST, ALT, TAG, Chol and VLDL were measured. Immunohistochemistry was performed for α-SMA and hematoxylin-eosin staining, Masson's trichrome and Syrian red. The hepatic expression of IL-6, TNFα, COL1A1 and TGF-β mRNAs was determined by qRT-PCR. Quantitative variables were analyzed with ANOVA, Tukey for parametric data and Kruskal-Wallis for non-parametric data. Opinion CI00518 of ethics and investigation committee.

Results: Animals at week 16 showed high body weight compared to animals with standard diet, presence of steatosis and liver inflammation ($p < 0.05$). Serum glucose increased at week 12 and 16 ($p < 0.05$). The weight of the liver and epididymal fat increases as the model is established, without achieving statistical significance. The histological parameters coincide with the establishment of a steatohepatitis, while the values of the biochemical parameters increase remarkably compared to the control group. Inflammatory and fibrotic genes increase at 16 weeks compared to the control group.

Conclusions: Exposure to a diet high in fat and simple sugars induced increased body weight, steatohepatitis, inflammation, hyperglycemia, and increased expression of liver enzymes and genes involved in inflammation and fibrosis.

Conflicts of interest: The authors have no conflicts of interest to declare.

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15

Development of a defatting strategy to reduce lipid accumulation and improve the viability of steatotic grafts in liver transplantation

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Background and aim: In order to reduce mortality on waiting list, therapeutic strategies are required to increase the use of steatotic liver grafts in transplantation. However, steatotic grafts tolerate poorly ischemia-reperfusion (I/R) injury, and therefore they show a very high risk to early allograft dysfunction or primary nonfunction after transplantation. The aim of the present research was to evaluate the potential of 3 pharmacological modulators of lipid metabolism to induce defatting and protection against hepatic damage during cold preservation period in steatotic liver grafts.

Material and methods: Wistar rats were fed with a high-fat diet to induce steatosis. Then, steatotic livers were preserved at 4° C for 6 hours, either in Custodiol preservation solution, or in Custodiol solution enriched with caffeine, choline, or L-carnitine. At the end of this period, grafts were washed-out and transaminases and triglycerides in liver tissue were determined. This study was approved by the institutional Research Ethics Committee.

Results: Addition of caffeine to Custodiol solution decreased hepatic triglycerides content by 56% in steatotic grafts when compared with grafts preserved only in Custodiol. Triglycerides content was similar in steatotic grafts preserved in Custodiol enriched with choline or L-carnitine, and in those grafts preserved in Custodiol without additives. Regarding liver injury, preservation in Custodiol supplemented with caffeine, choline or L-carnitine resulted in a decrease in transaminases, compared to the levels observed in preservation with solely Custodiol.

Conclusions: Addition of caffeine to preservation solution trigger defatting in steatotic liver grafts, which is associated with protection against I/R injury. The enrichment of preservation solution with choline or L-carnitine decrease I/R injury in steatotic grafts, but this effect was not related to reduction in triglyceride content.

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16

Hepatoprotective effect of sodium (*S*)-2-hydroxyglutarate against ischemia-reperfusion injury in Wistar rats

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Background and aim: Ischemia-reperfusion (IR) injury is one of the leading causes of early graft dysfunction in liver transplantation. Techniques such as ischemic preconditioning protect the graft through the activation of the hypoxia-inducible factors, which are the main regulators of oxygen homeostasis and are downregulated by the EGLN prolyl-hydroxylases. The inhibition of EGLN has a therapeutic effect against IR injury. Our aim was to evaluate the effect of the EGLN inhibitor sodium (*S*)-2-hydroxyglutarate [(*S*)-2HG] against liver IR injury in Wistar rats.

Material and methods: (*S*)-2HG was synthesized from L-glutamic acid by diazotization/alkaline hydrolysis, and its structure was confirmed by nuclear magnetic resonance. Thirty-one female Wistar rats were used, weighing 250 – 300 g, randomly divided in the following groups, following the specifications of the NOM-062-ZOO-1999: IR (n = 7, ischemia: 20 minutes, reperfusion: 60 minutes), sham (SH, n = 7, laparotomy without IR), non-toxicity (HGTox, n = 6, 25 mg/kg, *p.o.*, twice per day for two days, laparotomy without IR), and (*S*)-2HG + IR (HGIR, n = 7, same dose as HGTox group + IR induction). Serum levels of ALT, AST, LDH, ALP, glucose, and total bilirubin, were assessed. Tissue levels of IL-1β, IL-6, TNF-α, malondialdehyde, SOD, and glutathione peroxidase were also evaluated. This project was approved by the Ethics and Research Committee of our institution (Registration number: HI19-00003).

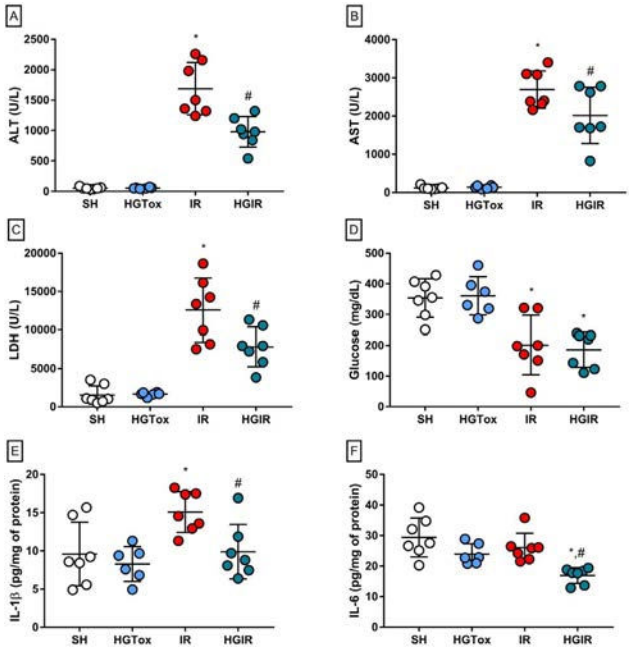

Figure. Liver injury and inflammatory biomarkers. (A) Serum alanine aminotransferase; (B) Serum aspartate aminotransferase; (C) Serum lactate dehydrogenase; (D) Serum glucose; (E) Tissue interleukin 1β; (F) Tissue interleukin 6. One-way ANOVA with Tukey *post hoc* test, $^{*}p < 0.05$ versus SH; $\#p < 0.05$ versus IR.

Results: A difference in the levels of ALT, AST, LDH, glucose, IL-1β, and IL-6 was observed among the groups (Figure). No hepatotoxic effect was observed when comparing the HGTox group versus the SH group. There were also no differences in the other biomarkers assessed.

Conclusions: (*S*)-2HG showed a hepatoprotective effect, decreasing the levels of liver injury and inflammation biomarkers. No hepatotoxic effect was observed at the tested dose.

Conflicts of interest: The authors have no conflicts of interest to declare.

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17

Cellular and molecular characterization of the pirfenidone effects on an hepatocarcinogésis experimental model

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Background and aim: Hepatocellular carcinoma (HCC) is a primary neoplasm of the liver with high recurrence and high mortality rate. The etiological factors are hepatitis B and/or C virus infections, non-alcoholic steatohepatitis, alcohol consumption, and aflatoxin b1 exposition. These factors promote inflammation, fibrosis, and cirrhosis, and alter the expression of genes and molecular mechanisms, initiating hepatocarcinogenesis. The modified resistant hepatocyte model (MRHM) has been established which simulates the stages of carcinogenesis. Pirfenidone (PFD) has shown antifibrotic, anti-inflammatory and antioxidant effects in liver damage models, so the aim was to evaluate the administration of PFD on histopathological alterations and the expression of key proteins in the development of hepatocarcinogenesis in MHRM.

Material and methods: Longitudinal experimental study. 30 Wistar rats were divided into 3 groups: control group, carcinogenic damage group, and carcinogenic damage group plus daily administration of PFD. The physical and clinical data of the animals were analyzed at 30 days. All tissues were subjected to H&E, and Masson trichrome histological assays, and analysis of proteins involved in liver fibrosis, acute and chronic inflammation, apoptosis, cell division, tumor promotion/suppression, and cell metabolism using Western-Blot tests and microscopy confocal. Experiments for triplicate were performed; data were analyzed and plotted in GraphPad Prism 7.

Results: Morphological analysis: damage group shows dense, pale brown and inflamed livers compared to control and PFD groups. PFD administration prevents damage in the hepatocyte architecture, reduces periportal fibrosis and prevents inflammation overexpression markers (NFkB, IL-6, and TNFalpha) and cell