Funding: The resources used in this study were from the hospital without any additional financing

Declaration of interest: The authors declare no potential conflicts of interest.

https://doi.org/10.1016/j.aohep.2022.100855

Pirfenidone regulates antioxidant response via NRF2 in an experimental model of hepatocellular carcinoma

HC Monroy-Ramírez[1], HO Miranda-Roblero[1], M Galicia-Moreno[1], A Santos-García[2], J Armendáriz-Borunda[1,2]

[1] *Institute of Molecular Biology in Medicine and Gene Therapy. University Center for Health Sciences (CUCS). Guadalajara University. Guadalajara. Mexico*
[2] *School of Medicine and Health Sciences. Tecnológico de Monterrey campus Guadalajara. Zapopan. Mexico*

Introduction and Objectives: 1) Determinate if pirfenidone (PFD) modifies oxidative stress markers. 2) Evaluate PFD effects on transcription factor Nrf2 signaling pathway.

Materials and methods: Eighteen Fischer-344 rats divided into three groups were used: 1) untreated (NT), 2) carcinogenic damage (HCC) generated by weekly administration of diethylnitrosamine (50mg/kg/week;i.p.) and 2-Acetylaminofluorene (25mg/ kg/wk, p.o) and 3) HCC treated with PFD (300 mg/kg, p.o.) (HCC/PFD) for 18 weeks. Histopathological analyzes of the liver were performed, MDA and GSH levels were quantified and SOD, CAT, GSTP1 and Nrf2 expression was evaluated by Western-Blot. Data were analyzed using ANOVA and Tukey's test as post hoc. The trial was approved by the research ethics committee.

Results: In the HCC group, Nrf2, SOD, CAT, and GSTP1 expression was increased. PFD treatment was effective in preventing the increase in MDA levels and allowed GSH increase; in addition, PFD was effective in modulating the expression of Nrf2 and antioxidant response proteins.

Discussion: Oxidative stress is key in the genesis of HCC and the mechanisms leading to antioxidant response are modulated by Nrf2. PFD is an antioxidant evaluated in several liver fibrosis models. Additionally, in this work, we have demonstrated that the antioxidant response of PFD in an HCC experimental model is mediated by Nrf2.

Conclusion: PFD delays the HCC development by regulating Nrf2 signaling pathway. Clinical studies with PFD are being devised to evaluate the safety.

Funding: The resources used in this study were from the hospital without any additional financing

Declaration of interest: The authors declare no potential conflicts of interest.

https://doi.org/10.1016/j.aohep.2022.100856

Dietary supplementation with methyl donors improves physiopathological conditions of NAFLD in a murine model

A Vazquez Esqueda, R Rosas Campos, S Rodríguez Sanabria, R de la Rosa Bibiano, J Armendáriz Borunda, A Sandoval Rodríguez

Institute of Molecular Biology in Medicine and Gene Therapy. Guadalajara University. Guadalajara. Mexico

Introduction and Objective: This study aimed to evaluate the benefits of supplementation with methyl donors of a diet rich in fat and sugars in a model of NAFLD.

Material and methods: Male mice of the C57BL/6J strain with an initial weight of 20-25g were fed with a conventional diet (ND n=8) or a diet high in fats and sugars (HF n=8) for 18 weeks; or with a diet rich in fats and sugars for 10 weeks, plus eight weeks of HF diet + supplementation with methyl group donors (HFMS n=8). At 18 weeks, ITT was performed; it was collected at sacrifice: liver, fat, and serum. Histological and biochemical analyzes were performed and global hepatic DNA methylation was quantified. The trial was approved by the research ethics committee.

Results: The supplemented animals (HFMS) showed a decrease in body weight, liver weight and epididymal and visceral fat ($p<0.001$). The area of the adipocytes in the HFMS group decreased significantly compared to the HF group. The HFMS group presented reduced serum levels of triglycerides and glucose and greater sensitivity to insulin. Histological analysis of livers from ND and HFMS animals showed no damage characteristic of NAFLD, such as lipid infiltration and inflammation. Global methylation increased in HFMS animals.

Discussion: Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver damage worldwide. The results in this work reinforce the evidence that supplementation with methyl group donor molecules could work as a therapeutic strategy to prevent the progression of the disease.

Conclusion: Supplementation with methyl donors of a diet high in fats and sugars has beneficial effects in a murine model of NALFD.

Funding: The resources used in this study were from the hospital without any additional financing

Declaration of interest: The authors declare no potential conflicts of interest.

https://doi.org/10.1016/j.aohep.2022.100857

Effects of in vitro lipid overload on LX-2 hepatic stellate cells

A Campos-Espinosa, G Gutiérrez-Reyes, C Guzmán

Liver, Pancreas and Motility Laboratory. Experimental Medicine Unit. Medical Faculty. UNAM/ General Hospital Mexico. Mexico City. Mexico

Introduction and Objectives: Studying lipid overload repercussions on hepatic stellate cells (HSC) is of great importance due to their role in fibrosis during NAFLD. Steatogenic cell culture of HSC is intended to elucidate pathogenic mechanisms in NAFLD.

Materials and Methods: LX-2 HSC were cultured in standard DMEM. Steatogenic medium was prepared: mild steatosis (MS:50μM sodium oleate/sodium palmitate (OA/PA) at 2:1 ratio), severe steatosis (SS:500μM 2OA:1PA). Control (C) was cultured in DMEM. Cells were pre-incubated in DMEM at standard conditions for 24h the incubated in MS or SS medium. Cells were incubated for up to 72h. Viability and mortality ratios were assessed; cellular proliferation and senescence were assessed. Data: Mean ± SD, two-way ANOVA followed by Tukey. $P<0.05$.

Results: Cell viability in MS significantly diminished by 13.6% at 72h, whereas SS showed 49.6 % lower viability from 48h compared with C. Regarding mortality rate, it was increased by 16.0% in MS from 72h and by 50.0% in SS from 48h compared with C. Proliferation was increased in both MS and SS at 24h and significantly decreased by 72h compared with C. Cellular senescence in both steatogenic conditions was diminished among 1.8-22.4% compared with C at 24 and 48h.

Conclusion: Steatogenic conditions induced an increased proliferation and lower senescence in LX-2 HSC at 24h in both MS and SS groups. These findings suggest that HSC might turn into an activated state. Our results agree with other reports showing that HSC activation and transdifferentiation increase their proliferation, avoiding

other cellular processes, including senescence while contributing to the pathogenesis of NAFLD.

Funding: This work was funded by CONACYT (CB-221137)

Declaration of interest: The authors declare no potential conflicts of interest.

https://doi.org/10.1016/j.aohep.2022.100858

Moringa oleifera decreases biomarkers of oxidative stress in a murine NASH model

A Monraz-Méndez[1], A Monraz-Méndez[1], R Escutia-Gutiérrez[1], JS Rodríguez-Sanabria[1], L Sánchez-Orozco[1], J Armendáriz-Borunda[1,2], A Sandoval-Rodríguez[1]

[1] *Instituto de Biología Molecular en Medicina y Terapia Génica, Centro Universitario de Ciencias en la Salud, Universidad de Guadalajara, Guadalajara, Jalisco, México*
[2] *Tecnológico de Monterrey, Escuela de Medicina y Ciencias de la Salud, Guadalajara, Jalisco, México*

Introduction and Objectives: This study aimed to evaluate the effect of Moringa oleifera aqueous extract on biomarkers of oxidative stress in a murine model of NASH.

Material and Methods: The characterization of the extract was performed by DPPH and ABTS spectrophotometric assays. Male C57BL/6J mice were randomly separated into two groups. 1) Standard diet (ND) (n = 5) (18% lipid) and 2) High fat (HF) diet (n = 10) (60% lipid and 42 g/L sugar in water of use), for 16 weeks. At the end of eight weeks, five HF group mice were divided into a subgroup, 3) Moringa Oleifera (HF + MO), 290 mg/kg/day p.o. for eight weeks. Malondialdehyde (MDA) levels were determined in liver homogenates and the transcriptome by microarray. Differences between groups were determined by ANOVA/Kruskal-Wallis test. The trial was approved by the research ethics committee.

Results: Moringa aqueous extract showed antioxidant capacity; DPPH values were 10081.4 0.3 and 22960.4 0.3 for ABTS. Hepatic MDA levels were increased in the HF group compared to the ND group ($p<0.05$) and decreased in the moringa-treated group ($p<0.05$). In the transcriptome, mRNAs involved in endoplasmic reticulum stress were underexpressed.

Discussion: An increase in MDA has been demonstrated in a murine model of NAFLD induced by a high-fat diet. In our study, Moringa administration reduces MDA production and gene expression of molecules involved in oxidative stress.

Conclusions: MO treatment is a therapeutic alternative for the NASH spectrum of liver disorders.

Funding: The resources used in this study were from the hospital without any additional financing

Declaration of interest: The authors declare no potential conflicts of interest.

https://doi.org/10.1016/j.aohep.2022.100859

Pirfenidone ameliorates MAFLD by improving insulin sensitivity and reducing epididymal fat

J. Gutiérrez-Cuevas, D López-Cifuentes, AS Sandoval-Rodríguez, AO Vázquez-Esqueda, JS Rodríguez-Sanabria, J Armendáriz-Borunda

Department of Molecular Biology and Genomics, Institute for Molecular Biology in Medicine and Gene Therapy, CUCS, University of Guadalajara, Guadalajara, Jalisco, Mexico

Introduction and Objectives: Metabolic associated fatty liver disease (MAFLD) is characterized by hepatic steatosis with the following three metabolic conditions: obesity/overweight, diabetes and metabolic dysregulation, either alone or in combination. Pirfenidone (PFD) has anti-inflammatory, antioxidant, and anti-fibrotic effects. The aim of this study was to investigate the effects of PFD in mice with MAFLD induced by high-fat/high-carbohydrate (HFHC) diet.

Materials and methods: At the age of 6-7 weeks, six male C57BL/6 J mice were fed with a normal diet (ND, 18% kcal from fat food) and twelve with HFHC (60% kcal from fat food and drinking water with 42 g/L of carbohydrates: 55% fructose and 45% sucrose) diet for 20 weeks; at 10 weeks of feeding, six mice with HFHC diet were administered PFD (300 mg/kg/day) by gavage. An insulin tolerance test was performed, and data analysis were performed using SPSS. The trial was approved by the research ethics committee.

Results: All HFHC mice showed an increase in body weight and visceral fat accumulation ($P<0.01$), including elevated fasting glucose at week 20 ($P<0.001$). Liver weight and liver/body weight ratio exhibited no statistical significance. HFHC mice intervened with PFD showed reduced body weight gain ($P=0.054$) and epididymal fat pad weight ($P<0.05$). PFD also improved insulin resistance.

Discussion: Obesity, systemic insulin resistance, and diabetes are commonly associated with MAFLD, which may progress to nonalcoholic steatohepatitis (NASH). PFD has been shown to have benefits in models of lipotoxicity and NASH.

Conclusions: PFD could be a promising drug for the prevention and treatment of MAFLD induced by obesity.

Funding: The resources used in this study were from the hospital without any additional financing

Declaration of interest: The authors declare no potential conflicts of interest.

https://doi.org/10.1016/j.aohep.2022.100860

Hepatoprotective effect of caffeine against ischemia-reperfusion damage

D Garza-Guzmán, L Torres-González, GR Govea-Torres, JM De la Rosa-García, CA Jiménez-Torres, DP Moreno-Peña, DR Rodríguez-Rodríguez, LE Muñoz-Espinosa, E Pérez-Rodríguez, P Cordero-Pérez

Liver Unity. Department of Internal Medicine. Universitary Hospital "Dr. José E. González". Nuevo León Autonomous University. Mexico

Introduction and Objective: This study aimed to determine the hepatoprotective effect of caffeine against Ischemia-Reperfusion (IR) damage in Wistar rats.

Material and methods: Eighteen female Wistar rats were divided into three groups (Sham, IR, Caffeine+IR, n=6). Hepatic ischemia was induced at 70% with 1 and 2 hours of reperfusion. The vehicle (saline solution) or 20 mg/kg of caffeine was administered before the induction of IR. The hepatoprotective effect was evaluated with biochemical markers, relative expression of genes associated with oxidative stress and inflammation, proinflammatory cytokines, and histology. The trial was approved by the research ethics committee.

Results: Caffeine significantly reduced levels of ALT, AST and direct bilirubin vs. IR group. Regarding the relative expression of genes, a significant decrease in the expression of the GPX, NF-$\kappa\beta$ and IL-1β genes was observed in the group treated with caffeine, while there was a decrease in the concentrations of IL-Iß, IL -6 and TNF-α;