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

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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.

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

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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.

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

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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