Materials and Methodology: Huh 7 cells were transfected with an empty vector, and 0.5 - 1.0μg of pNluc-NS5A-HCV or pCore-HCV plasmids for 24 - 48h and then proteins and RNA were extracted. NS5A protein expression was measured by NanoLuc activity. Core, Snail, TGFβ1 and E-cadherin protein levels were evaluated by Western Blot. NS5A and Core transcripts were quantified by RT-qPCR. Relative expression of SNAI, TGFβ1 and CDH1 was calculated in relation to ACTB and GAPDH endogenous expression.

Results: Viral NS5A and Core proteins were expressed in transfected Huh 7 cells. Transient transfection with pNluc-NS5A-HCV upregulated snail expression (4-fold) but downregulated E-cadherin expression (0.6-fold) at 24h. In addition, upregulated TGFβ1 expression (4-fold) and downregulates E-cadherin expression (0.7-fold) at 48h compared to the control. Meanwhile, transfection of pCore-HCV for 24h upregulated snail expression (2-fold); in contrast, it downregulated E-cadherin expression (0.3-fold) compared to control at 48h.

Discussion: Snail and TGFβ1 upregulation and E-cadherin downregulation had been associated with HCV infection in other studies. Our results suggest that HCV NS5A and Core have a direct role in EMT.

Conclusion: Hepatitis C virus regulates epithelial-mesenchymal transition biomarkers by NS5A and Core proteins expression in hepatoma cells.

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Diethylnitrosamine and 2-acetylaminofluorene chronic administration leads to biochemical and histologic changes related to hepatocellular carcinoma in Wistar rats

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Introduction and Objectives: This study aimed to analyze the biochemical and histological alterations produced in a model of chemical hepatocarcinogenesis by the chronic administration of diethylnitrosamine (DEN) and 2-acetylaminofluorene (2-AAF) in Wistar rats.

Materials and methods: Twelve Wistar rats weighing 180 to 200 g were divided into control and damage groups: rats were treated with DEN (50 mg/kg/wk) i.p and an intragastric dose of 2-AAF (25 mg/kg/wk) for 18 weeks. Serum clinical biochemistry was performed on VITROS Chemistry System 350® equipment. Masson's trichrome and Hematoxylin-Eosin stains were performed on the liver tissue. The trial was approved by the research ethics committee.

Results: The damage group had significant increases in total cholesterol, HDL-C, AST, ALT, ALKP, and GGT. Furthermore, histological analysis showed the loss of normal liver architecture with nuclear pleomorphism in the hepatocytes, atypical mitosis, and fibrous septa distributed between portal triads and collagen fibers through the hepatic sinusoids.

Discussion: Hepatocellular carcinoma models are a valuable tool to identify alterations during the progression of the disease. The Fischer-344 strain is frequently used in chemical hepatocarcinogenesis models since this strain shows greater susceptibility to the development of liver tumors. The damage induction model used in this work causes advanced hepatocellular carcinoma in Wistar rats, in spite of being a strain with intermediate susceptibility to hepatocarcinogenesis. The damage was evidenced by the presence of hepatomegaly, fibrosis, abundant nodules, histological changes, and biochemical alterations.

Conclusion: Chronic administration of DEN and 2-AAF induces characteristic alterations of hepatocellular carcinoma in Wistar rats.

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In vitro evaluation of the antifibrogenic effect of tamsulosin during its interaction with activated stellate cells

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Introduction and Objective: Liver cirrhosis is a chronic disease that affects one-fifth of the world; in Mexico, it is the third cause of mortality. It is caused by the uncontrolled production of the extracellular matrix. Attempts have been made to develop treatments that can reverse this disease, attention has been paid to the stimuli responsible for the activation of hepatic stellate cells (HSC), and the presence of adrenoreceptors in these cells has also been demonstrated. This study aimed to demonstrate in the present work a possible treatment with a neuroimmune activity that decreases the fibrogenic capacity of HSC.

Material and Methods: Rat's HSC in a quiescent state and activated by primary culture were used. Cells in a quiescent state contain retinol and lose it by activation. The degree of activation was assessed by immunofluorescence for α-SMA and cytochemistry with Oil Red. Cell proliferation was assessed by the MTT reduction technique. Norepinephrine was used to activate adrenergic signaling and tamsulosin was used as an antagonist of this pathway.

Results: Initially, we standardized the primary culture of HSC, identified by the α-SMA marker at seven days of culture. Subsequently, it was demonstrated that Noradrenaline treatment activated stellate cells due to the progressive increase of α-SMA and its proliferation. Moreover, tamsulosin treatment was shown to decrease retinol loss by preventing its activation and reducing proliferation.

Conclusion: Tamsulosin has a direct effect on decreasing the activity of quiescent and activated HSCs.

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Assessment of cellular proliferation, death and senescence in a model of steatosis in vitro

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Introduction and objectives: Hepatocyte cell culture in steatogenic medium is a useful, reproducible tool. During NAFLD, cells undergo processes in response to the steatogenic input. Here we aimed to study cellular proliferation, death, and senescence in an in vitro model of steatosis.

Materials and Methods: HepG2 hepatocytes were cultured in standard RPMI1640. Steatogenic medium was prepared by supplementing RPMI1640 with lipids in two levels: Mild steatosis (MS: 50 μM sodium oleate/sodium palmitate (OA/PA) at 2:1 ratio) and Severe steatosis (SS: 500 μM OA/PA 2:1). A control (C) group (RPMI1640) was included. 105 cells per well were allowed to attach for 24 h in RPMI1640 at 37°C and 5% CO2, then incubated in MS or SS medium for up to 96 h. Steatogenic medium was refreshed daily. Viability and mortality rates were assessed, and proliferation and senescence were analyzed by commercial kits, followed by a morphometric analysis. All assays were performed in triplicates. Data: Mean ± SD. 2-way ANOVA followed by Tukey. $P<0.05$.

Results: MS and SS showed significantly lower cell viability versus C. Mortality rates were increased in MS and SS. Proliferation was significantly decreased in MS and SS compared with C. MS showed a significantly increased senescence from 48 h versus C, whereas in SS decreased compared with C and MS.

Conclusion: MS showed an increment in senescence compared with C and might be considered a mechanism aimed at avoiding damaged-cell proliferation. In contrast, SS showed an increased mortality rate and decreased senescence, suggesting activation of death pathways as a response to lipid overexposure.

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The administration of pirfenidone modifies the expression of JMJD2B in a murine model of NASH

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Introduction and Objectives: This study aimed to evaluate how the administration of pirfenidone modifies the expression of JMJD2B in a murine model of NASH.

Materials and methods: 4–5-week-old male C57BL/6J mice fed a high-fat diet for 16 weeks. Follow-up was done at 4, 8, 12 and 16 weeks. Serum glucose, animal weight, caloric intake, AST, ALT, TAG, Chol and VLDL were measured. The liver was weighed, as was the epididymal adipose tissue. Masson's trichrome hematoxylin-eosin staining was performed. Dual-channel microarrays were hybridized to the 22,000-gene version of the Mus musculus genome. Analyzed with adjusted P-values of <0.05 and Z-score values of >1.5 and <1.5 considered significant. Quantitative variables were analyzed with ANOVA, Tukey for parametric data, and Kruskal-Wallis for non-parametric data. The trial was approved by the research ethics committee.

Results: The animals achieved the body and biochemical parameters that demonstrate the development of NASH. The genes involved in epigenetic processes responsible for the development of NASH (SIRT1, SIRT2, JMJD1B) and, in particular, in JMJD2B; which found to have significantly different between the HFD vs. HFP and HFD vs. ND groups.

Discussion: JMJD2B is a histone methylation modulating enzyme, implicated in the development of NASH. In our trial, pirfenidone modulates the expression of JMJD2B, helping the recovery of liver function through epigenetic regulation in a murine model of NASH.

Conclusion: Pirfenidone appears to modulate epigenetic factors, supporting recovery from the disease.

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Evaluation of deubiquiyinase USP15 expression during HCV replication

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Introduction and Objectives: This study aimed to evaluate the USP15 protein expression in HCV replication in vitro.

Material and methods: Huh-7 cell line was transfected with pFKI plasmid that encodes for the non-structural HCV proteins; this expression is regulated by the T7 RNA polymerase promoter; therefore, prior to transfection, the cells were infected with Vaccinia Virus for T7 RNA polymerase expression. After 24 h of transfection, at 37°C and 5% CO2, total protein was extracted and quantified using the Bradford method. USP15 expression was evaluated by Western Blot assay using the antibodies for USP15, NS3-HCV and actin as a control.

Results: The expression of NS3 was found in the transfected cells; in addition, a decrease in the expression of USP15 was observed compared to the control without viral proteins.

Discussion: USP15 expression was shown to be downregulated in cells expressing HCV nonstructural proteins compared to control cells. A lower effect on USP15 expression was detected in the transfected cells compared to the HCV-replicon cells (positive control); this may be due to the low expression of NS3 in transfected cells. Therefore, we observed a decrease in USP15 expression dependent on NS3 expression. USP15 is known to regulate pathways including TLR signaling, RIG-I signaling, NF-kB, and IRF3/IRF7-dependent transcription to produce pro-inflammatory cytokines and type I interferons. Therefore it is important to elucidate the mechanisms involved in this regulation by HCV.

Conclusion: USP15 expression is decreased in the presence of HCV nonstructural proteins.