Regarding SADR, there was a higher number in the GLE/PIB group (14) vs. SOF/VEL group (4) ($p<0.05$). 84% (16/18) of patients with SADR had a multi-DDI profile. 13% of total multi-DDIs patients showed SADR; GLE/PIB group showed SADR in 18% (13/71) vs 6% (3/52) in SOF/VEL group ($p<0.05$). Most SADR were reported in statin group, percentage higher in the GLE/PIB group vs. SOF/VEL group ($p<0.05$).

Both pDAAs showed a similar percentage of patients restarting a new pDAA within six months after the end of treatment (1.0% and 1.1%, respectively, p=NS).

Conclusions: In Spain, about 10% of HCV patients taking ≥ 2 comedications are at risk of multiple DDI with pDAAs. The potential risk of increased comedication as DDI outcome and the presence of suspected adverse reactions were higher in GLE/PIB in comparison with SOF/VEL.

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P- 25 ANTIOXIDANT EFFECT OF MORINGA OLEIFERA IN A MURINE MODEL OF NONALCOHOLIC STEATOHEPATITIS

Alejandra Monraz-Méndez[1], Raymundo Escutia-Gutiérrez[1], Jonathan Rodríguez-Sanabria[1], Ricardo De la Rosa-Bibiano[1], Laura Sánchez-Orozco[1], Arturo Santos[2], Juan Armendáriz-Borunda[1,2], Ana Sandoval-Rodríguez[1]

[1] *Institute of Molecular Biology in Medicine and Gene Therapy, University Center of Health Sciences, University of Guadalajara, Guadalajara, Jalisco, Mexico*
[2] *Monterrey Technological Institute, School of Medicine and Health Sciences, Guadalajara, Jalisco, Mexico*

Introduction and Objectives: One of the main mechanisms in the development and progression of nonalcoholic steatohepatitis involves oxidative and endoplasmic reticulum stress. Several studies have reported therapeutic effects of Moringa oleifera leaf extracts in different animal and cellular models due to their antioxidant, anti-inflammatory and lipid-lowering effects. This study aimed to evaluate the effect of Moringa oleifera aqueous extract on biomarkers of oxidative stress in a murine model of non-alcoholic steatohepatitis.

Material and methods: Characterization of the aqueous extract was performed by DPPH and ABTS spectrophotometric assays. Male C57BL/6J mice were randomized into two groups. 1) Conventional diet (ND) (n = 5) (18% lipid) and 2) High-fat diet (HF) (n = 10) (60% lipid and 42 g/L sugar in water of use) for 16 weeks. On the ninth week, five animals in the HF group 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 was measured by microarrays. miRNAs involved in liver disease were also determined. Statistical analysis was performed by differences between groups determined by ANOVA or Kruskal-Wallis test.

Results: Moringa aqueous extract showed antioxidant capacity; DPPH values were 10081.4 ± 0.3 and 22960.4 ± 0.3 for ABTS. Hepatic MDA levels increased in the HF group compared to the ND group ($p<0.05$) and decreased in the moringa-treated group ($p<0.05$). The transcriptome analysis demonstrated the downregulation of genes involved in endoplasmic reticulum stress. The miR-122-5p, miR-21a-5p, miR-34a-5p and miR-103-3p decreased in the MO-treated group.

Conclusions: Moringa oleifera treatment might be considered a therapeutic alternative for the NASH spectrum of liver disorders.

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P- 26 EFFECT OF PROTEIN X OF THE HEPATITIS B VIRUS AND HEXACHLOROBENZENE ON LIVER CELL GROWTH DYSREGULATION

Zahira Deza[1], Verónica Mathet[2], Lucia Coli[1], José Oubiña[2], Laura Alvarez[1], Ezequiel Ridruejo[1,3]

[1] *Laboratory of Biological Effects of Environmental Contaminants. Department of Human Biochemistry, School of Medicine, University of Buenos Aires. Buenos Aires. Argentina*
[2] *Research Institute of Medical Microbiology and Parasitology (IMPAM), UBA-CONICET. Buenos Aires. Argentina*
[3] *Chief, Hepatology Section, Department of Medicine. Center for Medical Education and Clinical Research "Norberto Quirno" (CEMIC). Buenos Aires, Argentina*

Introduction and Objectives: Chronic hepatitis B and exposure to persistent organic pollutants (COPs) can lead to cellular hepatocarcinoma (HCC), the most common liver tumor. HBV DNA encodes transactivator x, HBx protein. The HBx is required to initiate and maintain HBV replication. Hexachlorobenzene (HCB), COPśmember, is a promoter of hepatic preneoplastic foci. We have shown that HCB increases in rat liver PCNA, TGF-β1, VEGF and neo-angiogenesis in vivo models. This study aimed to analyze in vitro two models of HCC generation -associated with HCB or with the expression of HBx-.

Materials and Methods: The HCB effect on cell number (BrdU incorporation by Immunohistochemistry), PCNA (Western blot), TGF-β1 (RT-PCR) was studied in vitro in: 1.1) Huh-7; 1.2) Huh-7 transfected with HBx; 2) HepG2.2.15 (stable expression HBV) and 3) EA-hy926 (endothelial cell). In these last, an inhibitor of TGF-β1-RII (SB431542) was used. In 1.2, 2 and 3 used, 5μM HCB, 24h; in 1, we performed time (30, 60, 90 and 120) and dose (0,005; 0,05; 0,5 and 5μM) curves. Evaluated: a) PCNA protein levels, b) TGF-β1 levels and positive cell number/total cell.

Results: In Huh-7, TGF-β1 increased (20%, 69% and 78%, with 0.05, 0.5 and 5μM HCB, respectively) and PCNA (45% and 60%, with 0.5 and 5 μM HCB, respectively). In Huh-7/HBx, PCNA and TGF-β1 increased by 86% and 71%, respectively. In Huh-7/HBx and 5μM HCB, PCNA increased by 120% and TGF-β1 by 91%. In HepG2.2.15 PCNA was overexpressed by 76%. In EA-hy926, PCNA 29% and TGF-β1 by 43% increased. Both effects were prevented by pre-incubating endothelial cells with the specific inhibitor of TGF-B1 RII after HCB 5μM.

Conclusions: HCB and HBx induce cell proliferation in vitro. This effect is equivalent for both agents (HCB and HBx) and is enhanced by combining them. The proliferative effect is associated with TGF-β1 increase, which mediates the proliferation generated on both HCC and endothelial cell lines. These findings could partially explain the molecular mechanism involved in human HCC cell proliferation, disease progression and neo-angiogenesis.

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P-27 CELLULAR EFFECTS OF *IN VITRO* LIPID OVERLOAD ON HEPATIC STELLATE CELLS AND HEPATOCYTES.

Adriana Campos-Espinosa[1], José Luis Pérez-Hernández[2], Gabriela Gutiérrez-Reyes[1], Carolina Guzmán[1]

[1] *Laboratory of Liver, Pancreas and Motility, Experimental Medicine Unit, School of Medicine, UNAM/ General Hospital of Mexico. Mexico City, Mexico*

[2] *Liver Clinic, Gastroenterology Service, Hospital General de México. Mexico City, Mexico*

Introduction and Objectives: Hepatic cells undergo different processes in response to the steatogenic input of MAFLD. Hepatic cell culture in steatogenic medium is a useful, reproducible tool intended to elucidate these pathogenic mechanisms. This study aimed to study cellular proliferation, death, and senescence in hepatocytes and hepatic stellate cells (HSC) using a model of steatosis *in vitro*.

Materials and Methods: HepG2 hepatocytes were cultured in RPMI1640 (Control-Hep) and LX-2 HSC in DMEM (Control-LX2). Steatogenic media: either RPMI1640 or DMEM supplemented accordingly: *mild steatosis* (MS:50μM sodium oleate/sodium palmitate (OA/PA) at 2:1 ratio), *severe steatosis* (SS:500μM 2OA:1PA). HepG2 or LX-2 cells were preincubated for 24h at 37°C and 5% CO_2, then incubated in MS or SS medium for up to 72h. Steatogenic medium was refreshed daily. Viability, mortality, proliferation, and senescence were analyzed. Assays are performed in triplicates. Data: Mean±SD. 2-way ANOVA followed by Tukey. $P<0.05$.

Results: Hepatocytes: MS showed lower viability and proliferation, with increased mortality at 72h and higher senescence from 48h. SS displayed lower viability, and proliferation, with increased mortality but lower senescence from 24h. HSC: MS showed diminished viability and increased mortality (16.0%) at 72h. SS showed lower viability and increased mortality rate (50.0%) from 48h.

Proliferation increased in both MS and SS at 24h but decreased by 72h. Cellular senescence was diminished at 24 and 48h in both steatogenic conditions.

Conclusions: Steatogenic conditions induced different outcomes in the two cell lines studied. Hepatocyte behavior depends on lipid contents. In MS, increased senescence might be considered a mechanism to avoid damaged-cell proliferation. In SS, increased mortality rate and decreased senescence suggest lipotoxicity and activation of death pathways. In contrast, HSC cultured in steatogenic conditions might turn into the activated state, therefore increasing their proliferation and avoiding other cellular processes, including senescence. Both hepatocyte and HSC outcomes presented here contribute to the pathogenesis of MAFLD.

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P- 28 ATORVASTATIN SHOWS ANTI-PROMOTOR AND ANTI-NEOANGIOGENIC EFFECT IN HEPATOCELLULAR CARCINOMA DEVELOPMENT IN VIVO AND IN VITRO MODEL BY INHIBITING TGF-β1/pERK SIGNALING PATHWAY

Ezequiel Ridruejo[1,2], Zahira Deza[1], Giselle Romero Caimi[1], Lucia Coli[1], AndLaura Alvarez[1]

[1] *Laboratory of Biological Effects of Environmental Contaminants. Department of Human Biochemistry, School of Medicine, University of Buenos Aires. Buenos Aires, Argentina*
[2] *Chief, Hepatology Section, Department of Medicine. Center for Medical Education and Clinical Research "Norberto Quirno" (CEMIC). Buenos Aires, Argentina*

Introduction and Objectives: Hepatocellular carcinoma (HCC) represents 90% of liver tumors. Statins may reduce HCC incidence. Its antitumor activities are controversial and may be mediated by disrupting several hepatocarcinogenic pathways. This study aimed to evaluate *in vivo* and *in vitro* the anti-proliferative and anti-angiogenic action of atorvastatin (AT) in the development of HCC as well as its mechanisms of action.

Materials and Methods: *In vivo* model: the pesticide hexachlorobenzene (HCB) was used to promote the development of HCC in Balb/C nude mice inoculated with Hep-G2 cells. Tumor hepatic number, cell proliferation parameters (proliferating cell nuclear antigen, PCNA), cholesterol metabolism (3-hydroxy-3-methylglutaryl-coenzyme-A-reductase, HMGCoAR), angiogenesis and VEGF levels were analyzed. *In vitro* model: Hep-G2 and Ea-hy926 cells were used to evaluate the effect of AT (2,5; 5 and 5 mg/kg b.w.) on HCB-induced cell proliferation, migration, and vasculogenesis and analyze proliferative parameters.

Results: *In vivo*: AT 5 mg/kg prevented liver growth and tumor development and inhibited PCNA, TGF-β1 and pERK levels increase. AT 5 mg/kg prevented VEGF levels and skin blood vessel formation. *In vitro*, AT prevented cell proliferation and migration as well as tubular formation in the endothelial cell line by inhibiting the TGF-β1/p ERK pathway.

Conclusions: We were able to demonstrate the potential AT antiproliferative and anti-angiogenic effects in an HCC model and the involvement of TGF-β1 and pERK pathways.

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P-29 THE LIVER IN AMYLOIDOSIS: AN ANALYSIS OF THE INSTITUTIONAL AMYLOIDOSIS REGISTRY

María Adela Aguirre[1], Marcelina Carretero[2], Eugenia Villanueva[3], Elsa Mercedes Nucifora[4], María Soledad Saez[5], Erika Bárbara Brulc[4], Diego Pérez De Arenaza[3], Sebastián Marciano[6], María Agustina Marco[1], Gisela Bendelman[1], Patricia Beatriz Sorroche[5], María Lourdes Posadas Martínez[1,5]

[1] *Medical Clinic Service, Buenos Aires Italian Hospital, Buenos Aires, Argentina. Institute of Translational Medicine and Biomedical Engineering (IMTIB) Executing Unit of CONICET*
[2] *Internal Medicine Research Area, Medical Clinic Department, Buenos Aires Italian Hospital, Buenos Aires, Argentina*
[3] *Cardiology Department, Buenos Aires Italian Hospital, Buenos Aires, Argentina*
[4] *Hematology Department, Buenos Aires Italian Hospital, Buenos Aires, Argentina*
[5] *Institute of Translational Medicine and Biomedical Engineering (IMTIB) Executing Unit of CONICET. Non-Sponsored Research Area, Research Department, Internal Medicine Research Area, Medical Clinic Service, Buenos Aires Italian Hospital, Buenos Aires, Argentina*
[6] *Section of Hepatology, Buenos Aires Italian Hospital, Buenos Aires, Argentina*

Introduction and Objectives: The liver can be either compromised by infiltrative damage of amyloid, as it happens in AL and AA amyloidosis, or its cause, as it occurs in transthyretin TTR-related amyloidosis. In the latter, the liver synthesizes a defective variant TTR which has the capacity for cardiac, neurological, and renal damage, but the liver function is preserved. This study aimed to describe the clinical characteristics and prognosis of patients with liver involvement of amyloidosis (AL and AA)

Materials and Methods: Retrospective cohort of patients with hepatic involvement included in the Institutional Amyloidosis Registry (ClinicalTrials.gov NCT01347047) between June 2010 and January 2022. Clinical characteristics and complementary studies were analyzed, as well as their evolution.