

division activation. PFD increases apoptotic markers expression (Cas-3), tumor suppressors (p53) and re-establishes proteins in cellular metabolism regulation (PPARalpha/PPARgamma).

Conclusions: PFD administration prevents chemical-induced carcinogenic damage in MMRH. PFD decreases fibrotic and proinflammatory markers; likewise, PFD regulates tumor suppressor and mitogenic markers.

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Analysis of the molecular interaction of pirfenidone with PPAR-gamma and effects on the beta-catenine pathway in HEPG2 line



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Background and aim: PPARgamma is a nuclear receptor that regulates genes involved in energy metabolism. It consists of a transactivation domain at the N-terminus, two zinc fingers required for DNA binding, and a ligand-binding domain at the C-terminus that facilitates RXR-alpha binding and activation. The interaction of PPARgamma/beta-catenin has recently been established in type 2 diabetes and the development of colon cancer. On the other hand, Pirfenidone (PFD) has shown antifibrotic, anti-inflammatory, and antioxidant effects in various models of liver damage. The objective of our work was to demonstrate by *in silico* analysis that PFD is a ligand/agonist of PPARgamma and subsequently analyze the activity of beta-Catenin in the HepG2 hepatocarcinoma cell line.

Material and methods: Molecular interaction analysis was performed using the SwissDock platform, the images were made with the 3D UCSF CHIMERA processor. For *in vitro* analysis, the HepG2 cell line was used. The cells were treated with 500 μ M PFD, the non-selective agonist (GW7647; 100 nM) and the selective antagonist (GW9662; 100 nM) of PPARgamma for 24 hrs. Immunofluorescence and Western-Blot of PPAR gamma and beta-Catenin were performed. The experiments were carried out in triplicate, GraphPadPrism 7 was used to prepare the graphs and statistical analysis.

Results: *In silico* analysis shows that Pirfenidone binds to the Serine342 residue of PPARgamma, the same site that Rosiglitazone binds to. Immunofluorescence shows increased PPARgamma placement and lower beta-Catenin in the nucleus for cells treated with PFD and GW7647. The opposite is observed in control and GW9662-treated cells. There is a differential expression of PPARgamma and beta-Catenin in cells treated with PFD and GW7647.

Conclusions: PFD is a ligand /agonist of PPARgamma because it binds to the Serine342 residue, just as Rosiglitazone does (a pharmacological agonist used in the treatment of type 2 diabetes mellitus). Additionally, treatment with PFD in HepG2 cells decreases the translocation of beta-Catenin to the nucleus, which could contribute to slow the progression of HCC.

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Prolonged-release pirfenidone prevents myocardial fibrosis in a mouse nonalcoholic steatohepatitis model



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Background and aim: Obesity is associated with insulin resistance, nonalcoholic steatohepatitis (NASH) and myocardial fibrosis. Peroxisome proliferator-activated receptors (PPARs) regulate carbohydrate and lipid metabolism; improving insulin sensitivity, triglyceride levels, inflammation and oxidative stress. Pirfenidone has anti-inflammatory, antioxidant and antifibrotic effects. Aim, we investigated the molecular effects of prolonged-release pirfenidone (PR-PFD) in ventricular tissue of male C57BL/6J mice with NASH.

Material and methods: All experiments were performed in compliance with the guidelines of the bioterium-CUCS Research Committee at the University of Guadalajara and National Institutes of Health (NIH). Five-week-old mice were fed with normal diet (ND, 18% kcal from fat, $n=5$) and high-fat/high-carbohydrate (HFHC, 60% kcal from fat, plus 42 g/L: 55% fructose y 45% sucrose in water, $n=10$) diet for 16 weeks of feeding. At 8 week, five mice with HFHC diet were administered PR-PFD (350 mg/kg/day). We assessed insulin resistance, oil red o, hematoxylin-eosin, Masson's trichrome and picrosirius staining, western blot, immunohistochemistry, RT-qPCR and data by SPSS.

Results: Mice showed NASH with insulin resistance, myocardial steatosis and fibrosis, which were prevented by PR-PFD. Ventricular tissue of HFHC mice showed increased TNF- α , Nrf2, Desmin, Tgf β 1, Timp1, Collagen-I, Collagen-III, mRNA levels, including NF- κ B, Nrf2, α -SMA, Troponin-I, Acox1, Cpt1A and Lxr α protein levels compared to the ND ventricular tissues ($P \leq 0.05$). PR-PFD treatment decreased these genes overexpressed by HFHC diet ($P \leq 0.05$). PR-PFD overexpressed the Pgc1a mRNA levels and Ppar α , Ppar γ , Acox1 and Cpt1A protein levels ($P \leq 0.05$).

Conclusions: PR-PFD prevents the cardiac steatosis and fibrosis by sobreexpressing Ppar α , Ppar γ , Acox1 y Cpt1A proteins. PR-PFD is a promising drug for the treatment of cardiac fibrosis induced by NASH.

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