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Potential involvement of PPAR α activation in diminishing the hepatoprotective effect of fenofibrate in NAFLD
Accuracy of non- invasive panel in determining the stage of liver fibrosis in rats

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ABSTRACT

Background: Although Fenofibrate (FF) is a hypolipedmic drug and one of the PPARα agonists which is a drug target for non alcoholic liver disease (NAFLD), no studies had investigated its potential hepatic effects in such cases.
Aim: To compare between the effect of FF and Gemfibrozil (GF) on the prognosis of NAFLD in rats.
Methods: Sixty four rats were used and classified into two main groups. Group I (treated for 6 weeks): naïve, FF, GF groups and Group II (treated for 14 weeks and drugs were added at the last 6 weeks): Control, high fat diet (HFD) untreated, HFD + FF, HFD + FF + folic acid (FA) and HFD + GF groups. Body weight (BW), liver index (LI), renal perfusion test (RPT), glomerular filtration rate (GFR), serum creatinine (S.cr), plasma homocysteine (Hcy), liver function, non invasive markers of fibrosis and histopathology were done.
Results: HFD produced significant increase ($P < 0.05$) in BW, LI, S.cr, plasma Hcy, lipid profile and liver enzymes. It showed significant ($P < 0.05$) decrease in GFR and RPT. These findings were correlated to the histopathology. FF through its effect on GFR and renal function induced significant increase in plasma Hcy and that decreased its effectiveness in managing NAFLD associated with hyperlipidemia. The addition of FA improved significantly its hypolipidemic and hepatotoxic effects.GF showed none of the above FF effects and this may be due to its low affinity to PPAR α.
Conclusions: There is preference of adding FA to FF or using GF instead in cases of NAFLD. Moreover, this work implies the enhanced liver fibrosis (ELF) panel diagnostic performance in diagnosis of any and moderate degree of fibrosis in rats with NAFLD.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is one of the leading causes of chronic liver injury across the world and is also strongly related to other pathological conditions such as obesity, diabetes, cardiovascular diseases, metabolic syndrome [57] and expected to be the most frequent indication for liver transplantation by 2030 [7]. It consists of a spectrum of hepatic pathology starting from simple steatosis which means increase fat content of a normal liver from less than 5% of its weight to 50–80% in steatohepatitis (NASH), which may progress to cirrhosis and hepatocellular carcinoma [39].

It was suggested that peroxisome proliferator-activated receptor α (PPARα) activation might have protective and therapeutic effects against NAFLD [44]. Fenofibrate (FF) – PPARα agonist – is a hypolipidemic agent that has been widely used in the treatment of dyslipidemia [60]. In 2005, FF Intervention and Event Lowering in

Abbreviations: AUC, area under the curve; AUROC, area under a receiver operating characteristic; BW, body weight; CRNSS, Clinical Research Network Scoring System; ECM, extracellular matrix; ELF, enhanced liver fibrosis; FA, folic acid; FF, fenofibrate; FIELD, FF Intervention and Event Lowering in Diabetes; GF, gemfibrozil; GFR, glomerular filtration rate; H&E, hematoxylin-eosin; HA, hyaluronic acid; Hcy, homocysteine; HFD, high fat diet; ILs, interleukins; IPK, isolated perfused kidney; LI, liver; in, index; MMP, Matrix Metalloproteinases; NAFLD, Non-alcoholic fatty liver disease; NASH, non alcoholic steatohepatitis; NF-κB, nuclear factor κB; PIIINP, amino terminal propeptide of type III procollagen; PPAR, peroxisome proliferator-activated receptor; RPT, Renal perfusion test; S.cr, serum creatinine; SHR, spontaneously hypertensive rats; SREBPs, sterol regulatory element binding proteins; TIMP-1, tissue inhibitors of metalloproteinases-1; TNFα, tumor-necrosis factor-α.

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Diabetes (FIELD) study was performed to evaluate the effect of FF among the patients with type 2 diabetes mellitus and are at increased risk of cardiovascular disease, partly owing to dyslipidaemia. The result of this study showed disappointing reduction in cardiovascular events [34].

Liao et al. [37] and Mikael et al. [43] provided a link between plasma homocysteine (Hcy) which is elevated by FF and the disappointing reduction of cardiovascular events in the FIELD study. It was found that FF increases total plasma Hcy level by 55% compared with the placebo groups in clinical trials [17] but the molecular mechanisms were unknown and many theories have been suggested to explain that increase [18].

In 2011, Bruno et al. [5] mentioned that the effect of FF on glomerular filtration rate (GFR) may be the cause of increased the plasma homocysteine (Hcy). They referred the effect of PPARs agonists on the kidney to their natriuretic effect, which would activate the renin-angiotensin system and consequently decrease the GFR.

The effect of FF on the cardiovascular system was established and it has no role in our issue. We try to search about the effect of FF on liver homeostasis. In 1998, Fonseca et al. [23] mentioned that elevated Hcy is associated with insulin resistance (IR) which later was linked to the first hit theory for the pathogenesis of NAFLD.

In addition, Hcy-induced endoplasmic reticulum stress causes activation of both the unfolded protein response and sterol regulatory element binding proteins (SREBPs) [14], which leads to increased de novo lipogenesis as well as decreased β-oxidation dysregulation Recently, Dai et al. [13] has been working on the relationship between the degree of NAFLD and plasma concentration of Hcy. Interestingly, they correlate between hyperhomocysteinemia and the severity of liver damage. Homocysteine (Hcy) is an amino acid formed from methionine which in normal circumstances is converted to cysteine and partly remethylated to methionine with the help of folic acid (FA) and Vit B12 [51].

Accordingly, we hypothesized that the use of FF as hypolipidemic drug in patients with NAFLD may be hazardous and this risk may be alleviated by adding on FA or the use of another fibrates with less PPAR α agonistic effect such as gemfibrozil (GF).

2. Material and methods

2.1. Drugs and chemicals

Fenofibrate (FF) (Sigma Aldrich chemicals, Germany) was supplied as white powder, suspended in small amount of tween 80 and distilled water and was given with daily oral gavage at a dose of 100 mg/kg [12].

Gemfibrozil (GF) (Sigma Aldrich chemicals, Germany) was supplied as white powder, suspended in small amount of tween 80 and distilled water and was given with daily oral gavage at a dose of 100 mg/kg [47].

Folic acid (FA) (Sigma Aldrich chemicals, Germany) was supplied as white powder, dissolved in distilled water and was given with daily oral gavage at a dose of 10 mg/kg [30].

Cholesterol (Sigma Aldrich Chemicals, Germany) supplied as white powder. Bile salts (Sigma Aldrich Chemicals, Germany) supplied as brown powder. Commercial lard supplied as liquid oil.

2.2. Animals and grouping

The entire experimental protocol was approved by institutional ethical committee and utmost care was taken during the experimental procedure, as well as at the time of sacrifice following the principles of the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments.

2.2.1. Groups

Sixty four Wistar rats weighing from 150 to 200 g were purchased from the Holding company for Biological products and Vaccines (VACCERA), Helwan, Egypt. Rats were divided into non-high fat diet (HFD) and HFD groups (n = 24, 40 rats for non-HFD and HFD groups respectively). The duration of the experiment was 14 weeks. Rats were allowed at least one week to acclimatize to the lab conditions. A 12/12 h light/dark cycle was maintained with light on at 6 A.M. and off at 6 P.M., temperature was maintained at 25 °C. Animals were housed, each 4 animals in one cage. This study was carried out in the Department of Pharmacology and Medical Research Center, Faculty of medicine, Ain Shams University.

2.2.2. Non-HFD group

This group included rats not exposed to HFD (n = 8 for each subgroup). It was further subdivided into naïve or control group including rats fed standard chow diet and receiving vehicle (tween 80 and distilled water) orally for 6 weeks and FF and GF treated groups including rats fed standard chow diet and took either FF or GF for 6 weeks.

2.2.3. HFD group

This group included rats exposed to HFD. It was further subdivided into control group (n = 8) including rats fed standard chow diet for 14 weeks and HFD group (n = 32) including rats receiving HFD for 14 weeks. The later group was further subdivided into 4 groups (n = 8 for each group) including rats receiving orally either vehicle (tween 80 and distilled water) or FF 100 mg/kg/day or FF + FA or GF in the last 6 weeks of the experiment.

2.3. Induction of NAFLD [16]

A rat model of NAFLD was induced by feeding high fat diet for 14 weeks. The high fat diet consists of 72.8% ordinary chow, 2% cholesterol, 25% lard and 0.2% bile salts. Rats were allowed to feed ad libitum during the study.

2.4. Measurement of body weight (BW)

At 8 weeks and 14 weeks [9].

2.5. GFR: urine will be collected for 24 h by metabolic cage then creatinine will be measured in urine

- Urine flow rate (V) = volume collected/time duration
- The rate of excretion of creatinine = (V) × creatinine in urine
- GFR = creatinine excretion rate/serum creatinine concentration [55]

2.6. Biochemical studies

2.6.1. Samples collection and processing

Retro-orbital blood sample was withdrawn from each rat after 12 h fasting. Samples will be subsequently centrifuged for 15 min at 3000 rpm for separation of serum. Then serum will be immediately stored at (−80 °C) until used in the following biochemical assays:

2.6.1.1. Serum creatinine (S.cr). Their serum levels will be measured by automated spectrophotometric method using Synchron cx5 autoanalyzer (Beckman, USA)

2.6.1.2. Plasma homocystiene (Hcy). Plasma Hcy will be determined by using a corresponding enzyme-linked immunosorbent assay (ELISA) kit purchased from *RayBiotech, Inc.* (according to manufacturer instructions)