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Cilostazol attenuates indices of liver damage induced by thioacetamide in albino rats through regulating inflammatory cytokines and apoptotic biomarkers

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ABSTRACT

Even though cilostazol was assessed before in several models of atherosclerosis, so far its full systematic effect as a natural anti-inflammatory and anti-apoptotic mediator in the protection of liver damage and complication has not been fully clarified, which is the target of this study. For that purpose, we examined the protective effect of cilostazol (10 and 5 mg/kg, p.o. b.wt.) in an acute hepatic injury model by orally injecting it for 3 weeks prior to a single dose of TAA (300 mg/kg, i.p) injection. Ursodeoxycholic acid was used as a standard drug (50 mg/kg, p.o. b.wt.). After injection of thioacetamide by 48hr, rats were sacrificed. On the serum biochemical level, cilostazol ameliorated the thioacetamide consequence, where it presented a significant enhancement in the liver enzymes activities [Aspartate aminotransferase (AST) & Alanine aminotransferase (ALT)]. On the other hand, at the tissue level (Liver), it revealed a significant improvement in pro-inflammatory cytokines [Tumor necrosis factor alpha (TNF-α), Interleukin 1 beta (IL-1β), Nuclear factor kappa B (NF-κB), NF-κB (P65/P50 nucleus translocation), caspase-3, cleaved caspase-3 & C-reactive protein (CRP)], redox level [Reduced glutathione (GSH) & Malondialdehyde (MDA)], histopathological findings, Reverse transcription polymerase chain reaction (RT-PCR) analysis (expression of TNF-α and NF-κB mRNA levels), and immunohistochemical reaction (caspase-3 & TNF-α). Obviously, the high dose of cilostazol (10 mg/kg, p.o. b.wt.) displayed a more pronounced effect than its lower one and nearly equal to ursodeoxycholic acid in the most of the parameters. These results give a new awareness into the hopeful molecular mechanisms by which cilostazol attenuates several factors participated in the progression of liver damage.

1. Introduction

The liver is a highly vital organ, playing an important role in the metabolic functions. From the last years, the morbidity and mortality of many forms of liver diseases have increased all over the world (Trivedi and Hirschfield, 2013; Suk et al., 2014).

Cilostazol is a type III phosphodiesterase enzyme (PDE_3) inhibitor with partial type V phosphodiesterase (PDE_5) activity, which has been related to the elevation of the intracellular cyclic adenosine monophosphate (cAMP) level by preventing its hydrolysis (Kimura et al., 1985). Moreover, cilostazol reduces recurrent cerebral infarction (Gotoh et al., 2000) and mitigates ischemic brain injury post-transient focal cerebral ischemia by cyclic adenosine monophosphate response element binding protein (CREB) activator of progenitor cells and enhance hepatic blood flow and sinusoidal perfusion besides regulating pro-inflammatory cytokines (Akcan et al., 2006; Uchiyama, 2010).

Ursodeoxycholic acid is a secondary bile acid, which is a metabolic derivative of intestinal bacteria and it has anti-oxidative properties (Chun and Low, 2012). The positive effects of ursodeoxycholic acid in non-cholestatic liver injury is through preventing the damage of the liver mitochondrial functions and maintaining its structure in chronic alcohol toxicity (Lukivskaya et al., 2007). The mechanism of the ursodeoxycholic acid action could be related to the transposition of toxic bile acids from the bile acid pool as well as immunomodulatory and cytoprotective effects, and we used it in this study as a reference drug in evaluating cilostazol effects (Dilger et al., 2012; Kotb, 2012).

In this experiment, we decided to investigate the ability of cilostazol in combination with ursodeoxycholic acid in mitigating or restoring liver damage accompanied by inflammatory disorders, and complications.

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2. Material and methods

2.1. Drugs and chemicals

Thioacetamide was purchased from [Sigma-Aldrich Co., USA], while cilostazol (Pletal) from Otsuka Pharmaceuticals (UK) and ursodeoxycholic acid (Ursofalk) from MINAPHARM-Egypt. Cilostazol and ursodeoxycholic acid were administered orally, which they suspended in distilled water.

2.2. Animals

Mature male Wistar albino rats weighing 120–130 g were obtained from the National Research Centre Animal House (Dokki, Giza, Egypt) and were kept in a standard polypropylene cages under standard environmental conditions with equal light-dark cycles. Rats were adapted for 1 week and were fed rat normal pellet diet and water ad libitum, prior to the beginning of the experiment.

2.3. Ethics statement

This experiment was carried out in according to the recommendations in the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85–23, revised 1996) and under regulations of Animal Care and Use of National Research Centre in Egypt. All surgery was performed under deep sodium pentobarbital anesthesia and all efforts were made to minimize the suffering of animals.

2.4. Induction of liver damage and experimental design

60 rats were divided into 5 groups, the first group was fed normal diet and received the vehicle (distilled water) to be considered as normal control group. While, the second one received a single dose of thioacetamide (300 mg/kg, i.p) to induce a liver damage and is considered as control positive group (Mostafa et al., 2017). The third and fourth groups were administered cilostazol (5 and 10 mg/kg, p.o, respectively) for 3 weeks before the injection of a single dose of Thioacetamide (300 mg/kg, i.p) (Lee et al., 2010; Gokce et al., 2012), whereas the fifth group received ursodeoxycholic acid (50 mg/kg, p.o) for 3 weeks before the injection of a single dose of Thioacetamide (300 mg/kg, i.p) (Hatano et al., 2017). After injection of thioacetamide by 48hr, rats were fasted 18 h before killing to diminish the food-related variations in biochemical parameters levels.

2.5. Serum collection for analysis

Blood was collected from the retro-orbital venous plexus under sodium pentobarbital anesthesia and was centrifuged (700 × *g*, 4 °C, 20 min) to separate serum. Serum was used to determine liver enzymes by using colorimetric reagent kits (AST and ALT) (Quimica Clinica Aplicada, Spain).

2.6. Liver tissue extracts

After serum collection, rats were killed under a deep sodium pentobarbital anesthesia. The liver was separated out, washed, weighed and homogenized in phosphate buffer saline (PBS) [10%]. First part of the aliquot was centrifuged at 1500 × *g* at 4 C° for 15 min and the supernatant was collected and stored at −80C° for the direct assessment of certain parameters and the second part of the aliquot was exposed to two repeated freeze-thaw cycle to break the cell membranes, then centrifuged at 5000 × *g* for 5 min and stored at −80C° for the assessment of the other parameters.

2.7. Protein assay

The protein content in the liver tissue homogenates was analyzed using the Bradford technique and the bovine serum albumin was used as a standard (Bradford, 1976).

2.8. Assessment of liver parameters

ELISA rat kits were used for the estimation of TNF-α and IL1-1β (RayBiotech, USA), NF-κB (EIAab, China), NF-κB (P65/P50 nucleus translocation) (Cayman Chemical, USA), CRP level (IBL, Germany), MDA, GSH (CELL BIOLABS, USA), and total oxidant system (Dr. Franz Tatzber KFG, Austria). Also, caspase-3 (CUSABIO, China) and cleaved caspase-3 (DuoSet IC, USA) were estimated by ELISA rat kits.

2.9. Reverse transcription polymerase chain reaction (RT-PCR) assay

The number of the mRNA copies of NF-κB and TNF-α was assessed by quantitative RT-PCR in RNA extracts from hepatic tissue homogenates from rats of the all different groups. Total RNA was extracted from tissue using a QIA amp. RNA Mini kit Cat. No. 62341 (QIAGEN GmbH, Germany) as designated in the manufacturer's protocol. RT-PCR assays to specifically quantify rat NF-κB and TNF-α mRNA were carried out by using PCR fluorescence quantitative kits (SNP Biotechnology R& D Ltd., USA) and a detection system (Step one Plus™ Real-time device, Applied Biosystems, USA), according to the manufacturer's directions.

2.10. Liver histopathological examination

Different sections from different lobes of the liver control and treated rats were excised and fixed in 10% neutral buffer formalin. Then the tissue sections were dehydrated and embedded in paraffin blocks. Sections of 5 μm thickness were cut and stained with H&E then examined by light microscopy for routine histopathological examination. Ten liver sections per group were examined.

For assessment of hepatocellular degeneration and necrosis, a semi-quantitative lesion scoring was performed according to the method of Shirai et al., (2015) with some modifications, in which: grade 1 revealed mild vacuolar degeneration of hepatocytes and hepatocellular swelling was restricted to the centrilobular area; grade 2: extensive vacuolar degeneration of hepatocytes with diffuse hepatocellular swelling; grade 3: extensive vacuolar degeneration of hepatocytes and hepatocellular necrosis was confined to individual cell; grade 4: centrilobular necrosis was demonstrated in $^{<}$ 33% of tissue sections; grade 5: centrilobular necrosis was demonstrated in ≥ 33% of tissue sections. The obtained data were statistically analyzed.

2.11. Liver immunohistochemical examination

Immunohistochemical staining for a demonstration of caspase-3 and TNF-α immune reactivity was performed on formalin-fixed paraffin sections according to the method of Hegazy et al. (2016) and Filliol et al., (2016). The tissue sections were deparaffinized and incubated in 3% H_2O_2. Then the tissue sections were incubated with Rabbit polyclonal anti-caspase-3 and rabbit polyclonal anti- TNF-α (Bioss) as primary antibodies in Ventana automated machine (Ventana Medical Systems, USA). Diaminobenzidine (DAB) (Ventana Medical Systems, Tucson, AZ, USA) was used to demonstrate the immune reactive cells. Hepatocytes with brown cytoplasm were considered immune reactive cells. The Caspase-3 and TNF-α immune reactive cells were counted in five random high microscopic fields (40 ×) according to the method of Ibrahim et al., (2015). Then the obtained results were statistically analyzed.