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Cilostazol attenuates cholestatic liver injury and its complications in common bile duct ligated rats

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ABSTRACT

Cilostazol is a phosphodiesterase III inhibitor increases adenosine 3′, 5′-cyclic monophosphate (cyclic AMP) level which inhibits hepatic stellate cell activation. Its pharmacological effects include vasodilation, inhibition of vascular smooth muscle cell growth, inhibition of platelet activation and aggregation. The aim of the current study was to determine the effects of early administration of low dose cilostazol on cholestatic liver injury induced by common bile duct ligation (CBDL) in rat. Male Wistar rats (180–200 g) were divided into three groups: Group A; simple laparotomy group (sham). Group B; CBDL, Group C; CBDL rats treated with cilostazol (9 mg/kg daily for 21 days). Six rats from each group were killed by the end of weeks one and three after surgery, livers and serum were collected for biochemical and histopathological studies. Aspartate aminotransferase, alanine aminotransferase, gama glutamyl transferase, alkaline phosphatase and total bilirubin serum levels decreased in the cilostazol treated rats, when compared with CBDL rats. The hepatic levels of tumor necrosis factor-alpha, transforming growth factor-beta, and platelet derived growth factor-B were significantly lower in cilostazol treated rats than that in CBDL rats. Cilostazol decreased vascular endothelial growth factor level and hemoglobin content in the livers. Cilostazol significantly lowered portal pressure, inhibited ductular proliferation, portal inflammation, hepatic fibrosis and decreased hepatic hydroxyproline contents. Administration of cilostazol in CBDL rats improved hepatic functions, decreased ductular proliferation, ameliorated portal inflammation, lowered portal hypertension and reduced fibrosis. These effects of cilostazol may be useful in the attenuation of liver injury in cholestasis.

1. Introduction

Cholestasis is a syndrome resulting from disturbed bile formation. The etiology includes different diseases ranging from genetic defects in hepatocellular bile formation to inflammatory diseases of the bile ducts. Many cholestatic diseases are progressive and ultimately fatal (Sturm et al., 2009). Fibrosing cholestatic hepatitis is a less common but well-recognized severe complication of recurrent hepatitis C virus infection post-liver transplant (Al Nahdi et al., 2013). Intrahepatic accumulation of cytotoxic bile acids leads to cholestatic liver injury manifested by disruption of hepatocellular integrity, inflammation, fibrosis, cirrhosis and increased risk for development of cancer (Sturm et al., 2009).

Chronic hepatic inflammation leads to progressive liver fibrosis (Friedman, 2003). The key-process during progression of liver fibrosis is the synthesis and deposition of extracellular matrix (ECM), which mainly consists of different collagen types. The responsible cells for scar-formation in the liver are hepatic myofibroblasts, mainly deriving from hepatic stellate cells (HSCs). These are activated and stimulated by the ongoing inflammation in the liver which is associated with formation of profibrotic cytokines including transforming growth factor-β (TGFβ) (Friedman, 2008).

Platelet derived growth factor-B (PDGF-B) plays an essential role in wound healing and fibrosis (Lynch et al., 1987; Czochra et al., 2006). The PDGF-BB homodimer is a potent profibrogenic mediator in liver. PDGF-BB stimulates collagen production by portal fibroblasts and triggers hepatic stellate cell chemotaxis, which both contribute to peribiliary fibrosis (Kinnman et al., 2003). During cholestasis, the expression of PDGF-B increases in proliferating bile duct epithelial cells (BDECs) (Grappone et al., 1999). Potential inducers of PDGF-B expression in BDECs during cholestasis includes TGFβ, which contributes to liver fibrosis (Sullivan et al., 2010).

Neoangiogenesis and the development of an abnormal angioarchitecture in the liver are linked with progressive fibrogenesis (Lin et al., 2014). Cholangiocytes proliferate after common bile duct ligation (CBDL), these cells secrete Vascular endothelial growth factor (VEGF) (Chang et al.,2013). VEGF-induced HSCs and liver endothelial cells

Abbreviations: BDECs, Bile duct epithelial cells; CBDL, Common bile duct ligation; CCl_4, Carbon tetrachloride; FCH, Fibrosing cholestatic hepatitis; HSCs, Hepatic stellate cells; LECs, Liver endothelial cells; TBIL, Total bilirubin; VEGF, Vascular endothelial growth factor

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activation, accelerates hepatic fibrogenesis and angiogenesis, and hemodynamic dysarrangements in cirrhosis. VEGF targeting agents had been reported as potential drugs for cirrhosis (Lin et al., 2014).

Cilostazol is a phosphodiesterase III inhibitor increases Adenosine 3′, 5′-cyclic monophosphate (cyclic AMP) level. Increased intracellular cyclic AMP has been shown to inhibit HSC activation (Shimizu et al., 1999). Its pharmacological effects include vasodilation, inhibition of platelet activation and aggregation, and inhibition of vascular smooth muscle cell growth (Weintraub, 2006). The aim of the current study was to determine the effects of low dose cilostazol on cholestatic liver injury induced by CBDL in rat administered orally for one or three weeks. Liver function tests, Hepatic tumor necrosis factor alpha (TNFα), PDGF-B and TGF-β were analyzed. Hepatic angiogenesis was assessed indirectly by hemoglobin content and VEGF level. Liver fibrosis was determined by measuring of hydroxylproline contents. HSC activities were determined by immunohistochemical staining for alpha smooth muscle actin (αSMA).

2. Materials and methods

2.1. Animal

Adult male Wistar rats weighing between 180 and 200 g were used. The experimental protocols were approved by the Animal Care and Use Committee of Ain Shams University (Cairo, Egypt). The rats were housed in a specific pathogen-free (SPF) center, at room temperature of 24–26 °C and relative humidity of 60–65%. Water was provided ad libitum. The rats were well fed and housed for 3 days before any experimental protocols.

2.2. Reagents and drugs

Cilostazol was purchased from Sigma chemical (St. Louis, MO, USA). dimethylsulfoxide (DMSO) was used as a solvent for cilostazol.

2.3. Study design

2.3.1. Common bile duct ligated and treatment regimens in rat

Fifty two rats were divided into three groups: Group A; simple laparotomy group known as the sham group and administered DMSO by gavage as vehicle ($n=12$). Group B; Common bile duct ligated (CBDL), as previously described (Trebicka et al., 2010) and administered DMSO by gavage ($n=20$); Group C; CBDL rats treated with low dose cilostazol (9 mg/kg by gavage daily for 3 weeks) ($n=20$) (Gao et al., 2006). Six rats from each group were killed and liver and serum were collected by the end of weeks 1 and 3 after surgery. Sham-operated rats served as a control group.

2.3.2. Common bile duct ligation procedure

Laparotomy was performed under anesthesia with ketamine 100 mg/kg. The bile duct was isolated and double-ligated with a 3-0 silk suture. The abdominal wall and skin were closed with 4-0 silk sutures, and the antibiotic gentamicin (0.3 ml) was injected intramuscularly. Rats in the sham group underwent laparotomy with the bile duct isolated but not ligated (Trebicka et al., 2010).

2.3.3. Tissue and blood collection

Six rats from each group were killed after the described time points (end of week 1, 3). Blood was withdrawn to measure biochemical parameters using an automated analyzer. Serum samples were isolated using standard methods. Liver samples were cut into fragments and either snap-frozen in liquid nitrogen and stored at −80 °C or preserved in formaldehyde for histopathological and immunochemical assessment as described previously (Trebicka et al., 2010).

2.3.4. Preparation of liver homogenate

Snap-frozen liver tissues were homogenized using a BulletBlender tissue homogenizer (Next Advance) after thawing in phosphate buffered saline (PBS) containing protease inhibitors (1 protease inhibitor cocktail tablet per 10 ml buffer, 11836170001, Roche Applied Science). Liver tissue homogenized in 10 ml PBS per gram weight tissue. The homogenates were centrifuged at 10,000g for 30 min at 4 °C. The supernatants were used in assays of TNF-α, GF-β1, PDGF-BB, VEGF, hemoglobin and hydroxyproline contents without further freezing/thawing (Ma et al., 2011).

2.4. Biochemical analysis

2.4.1. Liver function tests

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), Gama glutamyl transferase (GGT), alkaline phosphatase(AP) and total bilirubin(T BIL) in the serum were measured using kits purchased from Diamond Diagnostic (Cairo, Egypt).

2.4.2. Measurement of hepatic angiogenesis-related factors

2.4.2.1. Determination of hepatic VEGF level. Hepatic level of VEGF was measured as an indicator of hepatic angiogenesis by a commercially available ELISA kit (BioAssay Systems), according to the manufacturer's instructions.

2.4.2.2. Determination of hepatic hemoglobin content. Hemoglobin content was measured using HLS-hemosafe reagent. The color obtained was measured colorimetrically at 530 nm.

2.4.3. Measurement of hepatic necrosis and fibrosis-related factors

Levels of hepatic TNF-α, TGF-β1 and PDGF-BB in the liver homogenate of rats were determined by using ELISA kit purchased from Ray Biotech, Inc.; Sigma-Aldrich Co. and R&D Systems (Minneapolis, MN), respectively. The procedures were performed according to the manufacturer's instructions.

2.4.4. Measurement of hepatic hydroxyproline

Hepatic hydroxyproline was determined using ELISA kit (BioAssay Systems).

2.5. Measurement of portal pressure

Rats in all groups were fasted for 12 h and anesthetized with an intraperitoneal injection of Phenobarbital sodium (50 mg/kg bodyweight). Median laparotomy was performed, and a small cannula (20G) irrigated with heparinized saline every 3 min was inserted into the distal part of the portal vein. The trocar of cannula was removed and the Miller Micro-tip catheter with a high fidelity pressure sensor and conductance electrodes was inserted into portal vein. This catheter was connected to Power Lab data acquisition systems (Power lab AD instrument) via the Ultra Pressure–Volume Unit .

2.6. Histopathological and immunochemical assessment

Livers (from 6 rats in each group) were collected at the end of the 1st and 3rd weeks of experiment. Right lobe of the liver was removed, cut into longitudinal sections 2–4 mm in thickness and kept in 10% formalin.

2.6.1. Staining

Hematoxylin and Eosin (H&E) and Masson's trichrome collagen staining: Liver inflammation, apoptosis and fibrosis were evaluated using the above stains.