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Taurine zinc solid dispersions enhance bile-incubated L02 cell viability and improve liver function by inhibiting ERK2 and JNK phosphorylation during cholestasis

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ABSTRACT

Dietary intakes of taurine and zinc are associated with decreased risk of liver disease. In this study, solid dispersions (SDs) of a taurine zinc complex on hepatic injury were examined *in vitro* using the immortalized human hepatocyte cell line L02 and in a rat model of bile duct ligation. Sham-operated and bile duct ligated Sprague-Dawley rats were treated with the vehicle alone or taurine zinc (40, 80, 160 mg/kg) for 17 days. Bile duct ligation significantly increased blood lipid levels, and promoted hepatocyte apoptosis, inflammation and compensatory biliary proliferation. *In vitro*, incubation with bile significantly reduced L02 cell viability; this effect was significantly attenuated by pretreatment with SP600125 (a JNK inhibitor) and enhanced when co-incubated with taurine zinc SDs. *In vivo*, administration of taurine zinc SDs decreased serum alanine aminotransferase and aspartate aminotransferase activities in a dose-dependent manner and attenuated the increases in serum total bilirubin, total cholesterol and low density lipoprotein cholesterol levels after bile duct ligation. Additionally, taurine zinc SDs downregulated the expression of interleukin-1β and inhibited the phosphorylation of Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase2 (ERK2) in the liver after bile duct ligation. Moreover, taurine zinc SDs had more potent blood lipid regulatory and anti-apoptotic effects than the physical mixture of taurine and zinc acetate. Therefore, we speculate that taurine zinc SDs protect liver function at least in part via a mechanism linked to reduce phosphorylation of JNK and ERK2, which suppresses inflammation, apoptosis and cholangiocyte proliferation during cholestasis.

1. Introduction

Cholestasis, characterized by abnormal accumulation of bile acids and bilirubin, is caused by structural and functional impairment of the hepatobiliary system and can occur in a number of clinical situations, including benign liver tumors, stricture of the bile duct, gallstone complications of pancreatitis and after biliary surgery. Patients with cholestasis develop serious complications such as sepsis, immune depression, coagulopathy, wound breakdown, gastrointestinal hemorrhage, and cardiovascular, hepatic and/or renal failure (Hofmann, 2002). Unfortunately, therapeutic strategies for this syndrome remain limited, not least because the mechanisms that mediate cholestatic liver injury are yet to be revealed. However, a number of key factors have been implicated in recent studies: cell death either by necrosis or apoptosis, disruption of the oxidative stress balance, inflammatory responses and bile duct epithelial cell proliferation (Glaser et al., 2009; Jones et al., 2015; Olteanu et al., 2012; Qi et al., 2015). Currently, ursodeoxycholic acid (UDCA) is the only FDA-approved drug for the treatment of cholestasis regardless of etiology, and is therapeutically administered to patients with primary biliary cirrhosis to increase bile flow and alter the hydrophobicity index of the bile acid pool (Marcinkiewicz and Kontny, 2014). However, the use of UDCA is costly and may lead to adverse events, including hepatitis, pruritus, cholangitis, ascites, vanishing bile duct syndrome, liver cell failure, severe watery diarrhea, pneumonia, dysuria, immune-suppression, mutagenic

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effects, withdrawal syndrome upon suddenly stopping treatment, and even death (Magd, 2012). Furthermore, the limited efficacy of UDCA in various cholestatic conditions necessitates the development of novel therapeutic agents.

Mammalian hepatocytes perform a myriad of functions, including the conversion of cholesterol to conjugated bile acids, followed by their secretion across the canalicular membrane into bile. At its simplest, bile acid formation can be considered the dominant chemical pathway for elimination of cholesterol. After their biosynthesis from cholesterol, C_{24} bile acids are linked via an amide linkage to glycine or taurine. This linkage is between the carboxyl group of the bile acid and the amino group of glycine or taurine. Linkage to glycine or taurine is termed N-acylamidation or simply "amidation," and glycine- and taurine-conjugated bile acids are referred to as amidates. The amide bond is highly stable and is not cleaved in tissues unless pathological levels of bile acids are reached. Conjugated bile acids are always fully ionized at physiological pH and therefore cannot permeate the apical cell membrane of hepatocytes, cholangiocytes or enterocytes. In addition, amidation renders bile acids soluble at acidic pH and resistant to precipitation by Ca^{2+} ions. As a result, high concentrations of bile acids can be maintained in the lumen of the biliary tract and small intestine. Cholestatic liver disease leads to a deficiency of bile acids in the small intestine. This in turn results in less efficient absorption of saturated fatty acids, an inability to absorb fat-soluble vitamins and may cause steatorrhea (Arias et al., 2009). Taurine has been shown to promote bile flow, increase the maximum bile acid secretion rate (González-Contreras et al., 2012) and prevent cholestasis induced by lipopolysaccharide, probably via inducing expression of the canalicular transporters multidrug resistance protein 2 and bile salt export pump 1 (Mühlfeld et al., 2003). In healthy adults, the proportion of taurine- and glycine-conjugated bile acids is 3:1 (Venturoni et al., 2012); however, this value varies between individuals and is influenced by the pool of hepatic taurine (Paauw and Davis, 1990). Additionally, and in contrast to non-conjugated or glycine-conjugated bile acids, taurine-conjugated bile acids exert a choleretic effect and prevent cholestasis (Wasserhess et al., 1993). Supplementation with taurine improves the hepatic activity of cholesterol 7-α-hydroxylase, the enzyme that limits the rate at which bile acids are synthesized (Van der Meer et al., 1988).

Several *in vivo* and *in vitro* studies have shown that oxygen-free radicals play a role in the pathogenesis of cholestatic syndrome (Cai et al., 2000; Jaeschke et al., 2002; Ohkawa et al., 1979; Rodrigues et al., 1998). One of the first studies to demonstrate that oxidative stress occurs in animal models of bile duct ligation (BDL) investigated lipid peroxidation in hepatic mitochondrial membranes by measuring thiobarbituric acid-reacting substances and lipid-conjugated dienes (Ohkawa et al., 1979). Since then, several studies have confirmed that oxidative stress occurs in BDL animal models (Copple et al., 2010; Jiang et al., 2012; Tain et al., 2013; Yang et al., 2015). Recent studies have suggested oxidative stress plays an important role in the pathogenesis of hepatic injury and induces hepatocyte apoptosis during cholestasis (Shafaroodi et al., 2010). Some of the earliest evidence for increased oxidative stress during cholestasis also came from studies in humans (Ros et al., 1984; Vendemiale et al., 2002). Vendemiale and colleagues demonstrated increased levels of markers of oxidative stress in patients with obstructive cholestasis: malondialdehvde (MDA) was elevated in the liver of patients with obstructive cholestasis, though liver total glutathione levels were reduced (Vendemiale et al., 2002). We previously synthesized solid dispersions (SDs) of taurine zinc at a 1:6 (w/w) ratio of the taurine zinc complex with polyvinylpyrrolidione (PVP) K30. Subsequently, we reported that these taurine zinc SDs enhanced the antioxidant defense system, partly through activating the transcription and synthesis of endogenous phase II metabolic enzymes and attenuating apoptosis via inhibition of JNK phosphorylation in an animal model of doxorubicin-induced liver injury (Wang et al., 2015).

Therefore, the overall objective of this study was to determine if taurine zinc SDs can prevent cholestatic liver injury. To address this objective, a well-defined model of cholestasis was established in Sprague-Dawley rats by double ligation and division of the common bile duct. SDs of the taurine zinc compound were administered to investigate if pretreatment with taurine zinc: i) could prevent the reduction in L02 cell viability observed during co-incubation with bile *in vitro*; ii) reduce hepatic apoptosis during cholestasis *in vivo*; and iii) regulate phosphorylation of the MAPKs to protect the liver against the development of cholestasis *in vivo*. The results of this study indicate that taurine zinc plays a critical hepatoprotective role following bile duct obstruction.

2. Materials and methods

2.1. Chemicals

Taurine was purchased from Zhongda Greenfield Biotech. Co. (Guangzhou, China). Taurine zinc and PVP in a ratio of 1:6 (w/w) were added to an alcohol solution to produce a suspension by cryogrinding under a nitrogen atmosphere (NS1001 High-Pressure Homogenizer, GEA Niro Soavi S.p.A. Inc., Parma, Italy). SDs of taurine zinc were produced using a spraydryer (Laiheng Scientific Instruments, Beijing, China). The operating parameters were: inlet temperature, 70 °C; outlet temperature, 50 °C; feed rate, 2–3 ml/min; atomization air pressure, 2 kg/cm^2 and inspiration, −280 mm WC. The molecular formula of the taurine zinc complex is shown in Fig. 1 (Shi, 2012). All other chemicals were of reagent grade.

2.2. Animals

Healthy male Sprague-Dawley rats (6–8 weeks-old; bodyweight, 180–220 g) were housed under a 12 h light/dark cycle at controlled temperature and humidity with free access to food and water. All procedures were carried out in accordance with guide lines approved by the Animal Ethics Committee of Sun Yat-Sen University (Guangzhou, China). All efforts were made to minimize the number of animals used and animal suffering during the experiments.

2.3. Experimental liver injury model

All surgical procedures were performed under anesthesia induced by intraperitoneal injection of 10% chloral hydrate (0.15 ml/kg, *i.p.*). The anesthetized animals were placed on a homoeothermic blanket system to maintain a constant body temperature, and the surgical area was covered with fluid-impermeable, self-adhesive drapes. A midline incision was made using sterile technique. After a midline laparotomy of 1–2 cm, the bile duct was exposed by caudal movement of the gut, doubly ligated using 5-0 silk sutures and transected at the level 0.7–0.8 cm

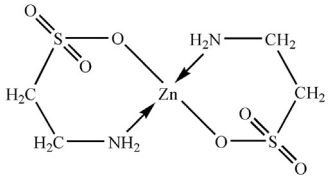

Fig. 1. Structure of taurine zinc complex compound.