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Protective effect of Et-1 receptor antagonist bosentan on paracetamol induced acute liver toxicity in rats ☆

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ABSTRACT

Paracetamol is one of the first rank drugs which cause hepatic damage during drug intoxications. Endothelin (ET) which is known as one of the most potent vasoactive agent has been shown to contribute in the pathophysiology of various diseases. We hypothesized that bosentan, which is a non-selective ET-1 receptor antagonist, can prevent liver damage. This study included 49 female rats. Groups; I: Healthy group, II: Paracetamol (2 g/kg orally). Groups 3, 4 and 5 received NAC 140 mg/kg (2 doses), BOS 45 mg/kg and BOS 90 mg/kg orally, respectively. 1 h after administration of pretreatment drugs, Groups 3, 4, 5 were given paracetamol. VI: received BOS 90 mg/kg. VII: received 140 mg/kg NAC (2 doses). According to biochemical results, TNF-α, ALT and AST levels were statistically increased in the paracetamol group, these parameters were improved in the bosentan groups. Paracetamol administration decreased SOD activity, GSH level and increased level of MDA in the liver, while bosentan administration significantly improved these parameters. In immunohistochemical staining ET-1 receptor expression was excessively increased in paracetamol group, but not in bosentan groups when compared to healthy control. All these results suggest that bosentan exerted protective effects against experimentally induced paracetamol toxicity in liver.

1. Introduction

Paracetamol has been frequently used as an over-the-counter and prescription with analgesic and antipyretic properties since 1983. Paracetamol has a reasonable safety profile if it is given at therapeutic doses (Black, 1980; Davidson and Eastham, 1966; Larson et al., 2005). However, when given at overdoses, paracetamol is the leading cause of liver, kidney, and other organ damages in both humans and animals (Wallace, 2004). Most of the paracetamol is rapidly metabolized in the liver by conjugation with glucuronic acid (40–67%) and sulfates (20–46%) (Hung, 2004). A small amount of paracetamol is metabolized in the liver by cytochrome P450 isoenzymes to the extremely toxic substance N-acetyl-p benzoquinoneimine (NAPQI). At over doses, the metabolite depletes hepatic glutathione stores (Jollow et al., 1974; Mitchell et al., 1973a, 1973b) and the increased levels of NAPQI covalently bound to membrane proteins in hepatocytes. This situation leads to the formation of reactive oxygen and nitrogen species, and initiates lipid peroxidation that eventually results in destruction, necrosis or apoptosis of the liver cells (Hinson et al., 2004; James et al., 2003; Nelson, 1990).

Several complex mechanisms were found to be involved in acetaminophen toxicity; oxidative stress (Ahmed and Khater, 2001; Srinivasan et al., 2001), nitric oxide (Beckman and Koppenol, 1996; Pryor and Squadrito, 1995), inflammatory mediators, such as tumor necrosis factor α (TNFα) and interleukin-1 (IL-1)(Gardner and Laskin, 2007; Laskin and Gardner, 2007; Laskin, 2009), however the main mechanism of paracetamol toxicity of in the liver is not fully understood. Therefore, new pathways on paracetamol toxicity have been continuously experimented.

Although N-acetylcysteine (NAC) is currently used for paracetamol toxicity (Brok et al., 2006), there is no effective cure for paracetamol toxicity. ET-1 has been known as the most potent and long-lasting vasoconstrictor agent since 1988 (Yanagisawa et al., 1988). ET-1 is produced Kupffer cells and hepatocytes (Gandhi

Abbreviations: ET, endothelin; NAC, N-acetylcysteine; PBS, phosphate buffered saline; TNF-α, tumor necrosis factor-α; ALT, alanine aminotransferase; AST, aspartate aminotransferase; SOD, Superoxide dismutases; GSH, Glutathione; MDA, Malondialdehyde; NAPQI, N- asetil- p- benzokinonimin; HIV, Human Immunodeficiency Virus; ELISA, enzyme-linked immunosorbent assay; CMC, carboximetilcelulos; BOS, bosentan; ANOVA, one-way analysis of variance; DMRT, Duncan's multiple range test; ROS, Reactive oxygen species; O.D., optical density.

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et al., 1990). The presence of ET-1 in the liver was demonstrated for the first time by Gandhi et al. (Gandhi et al., 1990). ET-1 has a variety of functions in many physiological and pathophysiological processes including the inflammatory process, overproduction of cytokines and free radicals. The increased expression of ET-1 have been shown to contribute to the pathological processes responsible for some liver diseases (e.g., cirrhosis, (Helmy et al., 2001)).

ET-1 receptors are divided into at least two types; ET-A (Cheng et al., 2005; Dhaun et al., 2009) and ET-B (Ehrenreich et al., 1990). The effects of ET-1 are blocked with the use of ET-1 receptor antagonists (Galie et al., 2004). Bosentan is commonly used as a nonselective ET-1 receptor antagonist (Clozel et al., 1993). Bosentan has been used in the treatment of pulmonary arterial hypertension (Siobal, 2007). In addition to the protective effects of bosentan have been demonstrated in specific studies including HIV, congenital heart disease and pediatric patients (Barst et al., 2003; Galie et al., 2006; Sitbon et al., 2004).

This study aimed 1: to investigate the possible role of ET-1 on paracetamol-induced hepatotoxicity in rats, 2: to observe the effects of bosentan at different doses administration on paracetamol-induced hepatotoxicity in rats, 3: to understand the relationship between the hepatoprotective mechanism of bosentan and antioxidant enzymes, oxidative stress parameters and TNF-α.

2. Materials and method

2.1. Chemicals

All chemicals for laboratory experimentation were purchased from Sigma Chemical (Germany). Paracetamol was purchased from Doga Ilac Hammaddeleri Tic. Ltd. Sti. (Turkey). Thiopental sodium was purchased from IE Ulagay A.S. Istanbul, Turkey and bosentan (Tracleer 125 mg Tb) was purchased from Actelion, US. N-acetylcysteine (Asist 200 mg capsules) was obtained from Husnu Arsan Drugs, Istanbul/Turkey.

Detection kits for superoxide dismutase activity (SOD), malondialdehyde (MDA), and glutathione (GSH) were purchased from Cayman and Cell Biolabs (USA), and Rat tumor necrosis factor-α (TNF-α) enzyme-linked immunosorbent assay (ELISA) kit was obtained from Invitrogen-KRC3011 (USA). The alanine aminotransferase (ALT) and aspartate aminotransferase (AST) reagent were purchased from USCN Life Science (China). The ET-1 antibody was purchased from ABCAM, Kimera Medical Laboratory Supplies, TURKEY.

2.2. Animals

Forty-nine (49) adult female Sprague Dawley rats ($n=7\times7$) weighing about 200–215 g were obtained from Medicinal and Experimental Application and Research Center (ATADEM). They were given standard rat pellet feed and tap water ad libitum. All animals were housed in stainless steel cages under standard laboratory conditions (light period 07.00 a.m. to 8.00 p.m., 21 ± 2 °C, and relative humidity 55%) throughout the experimental period. The animal care and experimental protocols were approved by the Experimental Animal Ethics Committee, Atatürk University, Erzurum, Turkey (26.10.2011/10).

2.3. Experimental design

Animals fasted overnight and were divided into seven equal groups ($n=7$). The effects of bosentan on paracetamol-induced hepatotoxicity were studied in rats. The first group served as a control and therefore did not receive any medication. Group 2 received only paracetamol, which was suspended in 1% CMC in 1× PBS 2 ml (per rat and administered orally at a dosage of 2000 mg/kg orally by gavage). Groups 3, 4 and 5 received N-acetylcysteine 140 mg/kg (second dose of N-acetylcysteine administration 12 h after received paracetamol.), bosentan 45 mg/kg (BOS 45) and bosentan90 (BOS 90) mg/kg orally by gavage in distilled water, respectively. One hour after administration of pretreatment drugs, Groups 3, 4, 5 were given paracetamol. Group 6 and 7 rats were treated with only BOS 90 mg/kg, NAC 140 mg/kg (second dose administration 12 h after received paracetamol.) orally by gavage, respectively. The rats were allowed food post-administration of drugs for the next 24 h until they were sacrificed.

The experimental groups can be summarized as follows:

Group 1: Intact
Group 2: Intact+2 g/kg paracetamol
Group 3: 140 mg/kg NAC (2 doses) +2 g/kg paracetamol
Group 4: BOS 45 mg/kg+2 g/kg paracetamol
Group 5: BOS 90 mg/kg+2 g/kg paracetamol
Group 6: BOS 90 mg/kg
Group 7: 140 mg/kg NAC (2 doses)

Following the experimental period, all rats were killed on the 24th hour after the administration of paracetamol by an overdose of a general anesthetic (thiopental sodium, 50 mg/kg). Blood samples were collected into heparinized bottles by heart puncture. The liver was removed immediately after sacrifice.

2.4. Biochemical analyses

2.4.1. Serum measurements of ALT, AST, TNF-α, and total protein

Serum samples were separated by centrifuging at 4000 rpm for 10 min at 4 °C within 1 h after collection, and were stored in a −86 °C freezer before being used for biochemical analysis (including ALT, AST, total protein, and TNF-α). To assess hepatic function, ALT and AST from each sample were measured in duplicate with highly sensitive ELISA kits specifically designed for rats, according to the manufacturer's instructions (USCN life science-E90207Ra and E91214Ra (China), respectively).

2.4.1.1. ALT measurements principles. The microtiter plate provided in this kit has been pre-coated with an antibody specific to **alanine aminotransferase (ALT)**. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated antibody specific to ALT. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added, only those wells that contain ALT, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme–substrate reaction is terminated by the addition of sulfuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of ALT in the samples is then determined by comparing the optical density (O.D.) of the samples to the standard curve.

2.4.1.2. AST measurement principles. The microtiter plate provided in this kit has been pre-coated with an antibody specific to **aspartate aminotransferase (AST)**. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated antibody specific to AST. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added, only those wells that contain AST, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme–substrate reaction is termi-