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Betaine supplementation mitigates cisplatin-induced nephrotoxicity by abrogation of oxidative/nitrosative stress and suppression of inflammation and apoptosis in rats

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ABSTRACT

Cisplatin is one of the most potent chemotherapeutic antitumor drugs used in the treatment of a wide range of solid tumors. Its primary dose-limiting side effect is nephrotoxicity. This study aims to investigate the effect of betaine supplementation on cisplatin-induced nephrotoxicity. A single intraperitoneal injection of cisplatin (5 mg/kg) deteriorated the kidney functions as reflected by elevated blood urea nitrogen and serum creatinine levels. Oxidative/nitrosative stress was evident in cisplatin group by increased renal thiobarbituric acid-reactive substances (TBARS), an indicator of lipid peroxidation, reduced renal total antioxidant status and increased renal nitrite concentration. Cisplatin resulted in a decline in the concentrations of reduced glutathione, glutathione peroxidase, catalase, and superoxide dismutase in renal tissues. Renal tumor necrosis factor-α (TNF-α) was also elevated. Expressions of nuclear factor-kappa B (NF-κB) and caspase-3 were up-regulated in renal tissues as indicated by immunohistochemical analysis. Histopathological changes were observed in cisplatin group. Betaine supplementation (250 mg/kg/day) orally via gavage for 21 days prior to cisplatin injection was able to protect against deterioration in kidney function, abrogate the decline in antioxidants enzymes and suppressed the increase in TBARS, nitrite and TNF-α concentrations. Moreover, betaine inhibited NF-κB and caspase-3 activation and improved the histological changes induced by cisplatin. Thus, the present study demonstrated the renoprotective nature of betaine by attenuating the pro-inflammatory and apoptotic mediators and improving antioxidant competence in kidney tissues of cisplatin treated rats. Betaine could be a beneficial dietary supplement to attenuate cisplatin nephrotoxicity.

1. Introduction

Betaine (trimethylglycine) is a natural component that is widely found in animals, plants, and microorganisms and rich dietary sources include seafood, especially marine invertebrates; wheat germ or bran; and spinach (Craig, 2004). It is rapidly absorbed and utilized as an osmolyte and a methyl donor due to the presence of three methyl groups in its structure and thereby helps to maintain liver, heart, and kidney health. Betaine insufficiency is associated with lipid metabolism disorder, diabetes, metabolic syndrome, and vascular diseases in patients (Lever and Slow, 2010). Betaine can reduce the risk for inflammation-related diseases via inhibiting the expression of IL-6 and TNF-α (Go et al., 2007; Lv et al., 2009). Betaine is an endogenous metabolite of choline that is needed for the structural integrity and signaling functions of cell membranes; it directly affects cholinergic neurotransmission, transmembrane signaling, and lipid transport/metabolism (Zeisel and Blusztajn, 1994). It plays an important role in homocysteine metabolism (Barak et al., 1996). Betaine supplementation is effective for reducing plasma homocysteine levels in humans (Steenge et al., 2003) and in homocystinuria patients with MTHFR deficiency (Smolin et al., 1981). Betaine generally appears to be safe at a daily intake of 9–15 g (average of

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12 g). Subacute and subchronic rat studies determined that betaine is nontoxic at all doses studied (0–5% of the diet) (Hayes et al., 2003).

Cisplatin (cis-diamminedichloroplatinum II, CDDP), is the most important platinum anticancer drug widely used in the treatment of head, neck, ovarian, and testicular cancers. Nevertheless, its full clinical utility is limited because of toxicity including renal and hepatic toxicity (Arany and Safirstein, 2003; O'Dwyer et al., 1999). 25–35% of patients experience a significant decline in renal function after the administration of a single dose of cisplatin (Ries and Klastersky, 1986). Nephrotoxicity is one of the most important side-effects of cisplatin therapy, affecting primarily the S3 segment of the proximal tubules (Townsend et al., 2003; Miller et al., 2010). The kidney accumulates cisplatin to a greater degree than other organs and is the major route for its excretion. Cisplatin selectively damage proximal tubular epithelial cells (Camano et al., 2010). Its concentration in proximal tubular cells is about five times the serum concentration (Kuhlmann et al., 1997), this disproportionate accumulation of cisplatin in renal tissue contributes to cisplatin-induced nephrotoxicity (Arany and Safirstein, 2003).

The mechanisms of cisplatin nephrotoxicity are complex and involve numerous processes as inflammation (Faubel et al., 2007), production of reactive oxygen, (Davis et al., 2001) and nitrogen species (Chirino and Pedraza-Chaverri, 2009), as well as cell apoptosis (Camano et al., 2010). Although the production of reactive oxygen species (ROS) by the cisplatin has been implicated in the pathogenesis of cisplatin-induced renal injury (Matsushima et al., 1998), the precise mechanisms underlying the disorders remain to be unknown and still a matter of debate. Moreover, efficient pharmacotherapies to attenuate this devastating complication of cisplatin chemotherapy are not available. Targeting and modulating the internal antioxidant mechanisms by chemopreventive agents has become a part of many therapeutic strategies. A large number of natural products and dietary components have been evaluated as potential chemoprotective agents. Nothing is known about the role of betaine in cisplatin-induced nephrotoxicity.

The aim of the present work is to investigate the effect of betaine supplementation on acute renal injury induced by cisplatin in rats hoping to accentuate the progression of renal injury as a side effect of cisplatin treatment. Efficacy of betaine supplementation against the nephrotoxicity was evaluated in terms of biochemical estimation of oxidative/nitrosative stress markers, antioxidant enzyme activities, inflammatory and apoptotic markers and histopathological changes.

2. Materials and methods

2.1. Chemicals

Betaine, cis-platin, Thiobarbituric acid, 5, 5-dithiobis-(2-nitrobenzoic acid) were purchased from Sigma St. Louis (Mo, USA). Blood urea nitrogen and creatinine were measured using kits from Biomérieux Inc., (France). Total Antioxidant Status (TAS), glutathione peoxidase, and superoxide dismutase were measured using diagnostic kits provided by Randox Chemical Co. (Antrim, United Kingdom). Chemicals used for measuring catalase and reduced glutathione were obtained from Sigma St. Louis (Mo, USA). Antibodies used for nuclear factor kappa-β and caspase-3 immunohistochemical studies were purchased from R& D System (MN, USA). Caspase-3 activity was measured using caspase-3 colorimetric assay (catalog number BF 3100) provided by R&D Company (MN, USA). TNF-α and nitrite/nitrate concentration were measured using enzyme-linked immunosorbent assay kits from R&D Systems (MN, USA).

2.2. Animals

Adult male Wistar rats, weighing 220–250 g, were used in this study. They were obtained from the Animal Care Centre, College of Pharmacy, King Saud University. All the animals were fed a standard rat chow and water *ad libitum* and kept in a temperature-controlled environment (20–22 °C) with an alternating cycle of 12-h light and dark. The animals used in this study were handled and treated in accordance with the strict guiding principles of the National Institution of Health for experimental care and use of animals.

2.3. Experimental design

The animals were divided into four groups of 10 rats each. The details of groups are: Group 1: saline control group which received the saline vehicle alone; group II: betaine group in which betaine (250 mg/kg/day) was given orally via gavage for 21 days; group III: cisplatin group in which rats were injected with a single intraperitoneal injection of cisplatin (5 mg/kg) and group IV: cisplatin + betaine group in which betaine (250 mg/kg/day) was given orally via gavage for 21 days before the single cisplatin injection and daily for 5 days after cisplatin. The doses of cisplatin and betaine were chosen depending upon the literature (Behling et al., 2006; Ganesan et al., 2010).

2.4. Estimation of renal function

Serum levels of blood urea nitrogen (BUN) and creatinine were estimated spectrophotometrically using commercial diagnostic kits (Biomérieux Inc., France).

2.5. Sample preparation for biochemical studies

At the end of experiments, the animals were sacrificed; blood and kidneys were collected.

Blood samples were collected and centrifuged for 10 min at 3000 rpm to obtain clear sera which were stored at −20 °C for subsequent measurement of renal functions. Kidneys were quickly excised, washed immediately with ice-cold physiological saline, blotted dry, and weighed. Portions were taken for histopathological and immunohistochemical studies and the remaining parts of kidneys were homogenized in ice-cold saline to produce 10% (w/v) homogenates, which were centrifuged at 1000*g* for 10 min at 4 °C. The supernatants were divided into aliquots and were kept at −80 °C until assayed for biochemical studies.

2.6. Biochemical assays for oxidative stress markers

Kidney tissues were homogenized in phosphate buffer (pH = 7) in a ratio of 1/10 (w/v) and centrifuged at 3000 × *g* for 5 min and the supernatant was used for biochemical assays.

2.6.1. Determination of total antioxidant status

Total Antioxidant Status (TAS) was determined using kit from Randox Company. The principle of the method is based on the generation of the ABTS radical cation (ABTS•+) from the interaction between metmyoglobin, 2,2′-azinobis-(3-ethyl-benzothiazoline-6-sulphonic acid) (ABTS) and a stabilized form of hydrogen peroxide. The TAS assay was performed using a 20 μl sample and assay read time of 3 min. Absorbance was measured at 600 nm. The results were expressed as mmol/min/mg protein.

2.6.2. Assessment of thiobarbituric acid-reactive substances concentration

The amount of renal thiobarbituric acid-reactive substances (TBARS) was measured by the thiobarbituric acid assay (TBA) as

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