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Betaine supplementation protects against renal injury induced by cadmium intoxication in rats: Role of oxidative stress and caspase-3

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ABSTRACT

Cadmium (Cd) is an environmental and industrial pollutant that can induce a broad spectrum of toxicological effects that affect various organs in humans and experimental animals. This study aims to investigate the effect of betaine supplementation on cadmium-induced oxidative impairment in rat kidney. The animals were divided into four groups ($n = 10$ per group): control, cadmium, betaine and betaine + cadmium (1) saline control group; (2) cadmium group in which cadmium chloride ($CdCl_2$) was given orally at a daily dose of 5 mg/kg body weight for four weeks; (3) betaine group, in which betaine was given to rats at a dose of 250 mg/kg/day, orally via gavage for six weeks; (4) cadmium + betaine group in which betaine was given at a dose of 250 mg/kg/day, orally via gavage for two weeks prior to cadmium administration and concurrently during cadmium administration for four weeks. Cadmium nephrotoxicity was indicated by elevated blood urea nitrogen (BUN) and serum creatinine levels. Kidneys from cadmium-treated rats showed an increase in lipid peroxidation measured as thiobarbituric acid-reactive substances (TBARS) concentration and reductions in total antioxidant status (TAS), reduced glutathione (GSH) content, glutathione peroxidase (GSH-Px) activity, superoxide dismutase concentration (SOD) and catalase activity. Caspase-3 activity, a marker of DNA damage was also elevated in renal tissues of cadmium-treated rats. Pre-treatment of rats with betaine substantially attenuated the increase in BUN and serum creatinine levels. Betaine also inhibited the increase in TBARS concentration and reversed the cadmium-induced depletion in total antioxidant status, GSH, GSH-Px, SOD and catalase concentrations in renal tissues. Renal caspase-3 activity was also reduced with betaine supplementation. These data emphasize the importance of oxidative stress and caspase signaling cascade in cadmium nephrotoxicity and suggest that betaine pre-treatment reduces severity of cadmium nephrotoxicity probably via antioxidant action and suppression of apoptosis.

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1. Introduction

Cadmium (Cd) is an abundant transition metal of worldwide concern because it accumulates in the environment as a result of its numerous industrial uses in electroplating, paints, dye stuffs, glass, metal alloys and batteries. Humans are exposed to cadmium, mainly through occupational and environmental contamination (Satarug and Moore, 2004). Non-occupational exposure to cadmium predominantly results from smoking, air pollution and consumption of cadmium contaminated seafood and water (Järup et al., 2000; Waisberg et al., 2003). Cadmium has an extremely long biological half-life of 15 years that essentially makes it a cumulative toxin in the liver and kidney (Ercal et al., 2001). The kidney is the critical target organ for cadmium-induced toxicity and is well documented by a number of studies in occupationally (Järup et al., 2000) and environmentally (Price et al., 1999) exposed human subjects, as well as in various experimental models (Ohta et al., 2000; Järup and Akesson, 2009). Cadmium can impair re-absorption of proteins, glucose, and amino acids and produce a variety of renal toxic effects involving the proximal tubules and glomerulus which is believed to be irreversible at an advanced stage (Ahn et al., 1999; Morales et al., 2006; Pari et al., 2007). Other effects of cadmium exposure are disturbances in calcium metabolism, hypercalciuria and formation of stones in the kidney (Hu, 2000). Occupational exposure has been linked to lung cancer and prostate cancer (Waalkes, 2000).

The deleterious effects of cadmium reported to date include increased generation of reactive oxygen species (ROS), altered antioxidant enzymes, modulation of apoptosis, and inhibition of DNA repair enzymes (Waalkes, 2003; Prozialeck and Edwards, 2012). Oxidative stress and reactive oxygen species (ROS) formed in the presence of cadmium could be responsible for its toxic effects in many organs (Wang et al., 2004; Watjen and Beyermann, 2004). Although several chelating agents and antagonists are established to reduce the cadmium toxicity, some of them are burned with undesirable side effects. Due to the intrinsic limitations and variability of efficacy of heavy metal chelating agents, cadmium intoxication therapy is looking for the development of new therapeutic agents with different mode of actions. Natural products have been the starting point for the discovery of many important modern drugs. A large number of natural products and dietary components have been evaluated as potential protective agents to reduce the toxicity of contaminating cadmium (Fouad et al., 2009; Prabu et al., 2010; Saïd et al., 2010).

Betaine (glycine betaine or trimethylglycine) is one of naturally occurring antioxidants which can be obtained from a variety of foods including wheat, shellfish, spinach, and sugar beets (Sakamoto et al., 2002). In humans, betaine is obtained from the diet or from its metabolic precursor choline (Zeisel et al., 2003). The physiologic function of betaine is either as an organic osmolyte to protect cells under stress or as a catabolic source of methyl groups via transmethylation for use in many biochemical pathways. As an osmolyte, betaine protects cells, proteins, and enzymes from environmental stress (e.g., low water, high salinity, or extreme temperature). As a methyl donor, betaine participates in the methionine cycle—primarily in the human liver and kidney where it converts homocysteine into methionine via betaine–homocysteine methyltransferase. In a renal context, betaine also plays a role in osmotic regulation in the kidneys, which are routinely exposed to high extracellular osmolarity during normal operation of the urinary concentrating mechanism (Craig, 2004; Kempson and Montrose, 2004). The ability of betaine to combat against oxidative stress has been demonstrated in many situations (Ozturk et al., 2003). Furthermore, dietary betaine has been shown to suppress nuclear factor-κB (NF-κB) and pro-inflammatory molecules such as cyclooxygenase-2 (COX-2) and inducible nitric oxide (Go et al., 2007).

Although reactive oxygen species (ROS) has been implicated in the pathogenesis of cadmium-induced toxicity, the protective effect of betaine against cadmium-induced nephrotoxicity was not yet investigated to the best of our knowledge. This study was carried out to examine the potential effects of betaine supplementation on cadmium-induced lipid peroxidation, oxidative stress, and nephrotoxicity in rats. In order to find out the exact underlying mechanisms of the protective action of betaine, the antioxidant activity and anti-apoptotic effect were determined.

2. Materials and methods

2.1. Chemicals

Betaine, thiobarbituric acid, 5,5-dithiobis-(2-nitrobenzoic acid) were purchased from Sigma (St Louis, MO, USA). Hydrogen peroxide was obtained from Aldrich (USA). Cadmium was purchased by Pfizer, USA. Blood urea nitrogen and creatinine levels were measured using kits from Biomérieux Inc. (France). Total antioxidant status, glutathione peroxidase, and superoxide dismutase were measured using diagnostic kits provided by Randox Chemical Co. (Antrim, United Kingdom). Caspase-3 activity was measured using caspase-3 colorimetric assay (catalog number BF 3100) provided by R&D Company (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 ($n = 10$ per group) as follow:

(1) Saline control group.
(2) Betaine group in which betaine (250 mg/kg/day) was given orally via gavage for two weeks.