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Original Article

Curcumin inhibits adenosine deaminase and arginase activities in cadmium-induced renal toxicity in rat kidney

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ABSTRACT

In this study, the effect of enzymes involved in degradation of renal adenosine and L-arginine was investigated in rats exposed to cadmium (Cd) and treated with curcumin, the principal active phytochemical in turmeric rhizome. Animals were divided into six groups ($n = 6$): saline/vehicle, saline/curcumin 12.5 mg/kg, saline/curcumin 25 mg/kg, Cd/vehicle, Cd/curcumin 12.5 mg/kg, and Cd/curcumin 25 mg/kg. The results of this study revealed that the activities of renal adenosine deaminase and arginase were significantly increased in Cd-treated rats when compared with the control ($p < 0.05$). However, co-treatment with curcumin inhibits the activities of these enzymes compared with Cd-treated rats. Furthermore, Cd intoxication increased the levels of some renal biomarkers (serum urea, creatinine, and electrolytes) and malondialdehyde level with a concomitant decrease in functional sulfhydryl group and nitric oxide (NO). However, co-treatment with curcumin at 12.5 mg/kg and 25 mg/kg, respectively, increases the nonenzymatic antioxidant status and NO in the kidney, with a concomitant decrease in the levels of malondialdehyde and renal biomarkers. Therefore, our results reinforce the importance of adenosine deaminase and arginase activities in Cd poisoning conditions and suggest some possible mechanisms of action by which curcumin prevent Cd-induced renal toxicity in rats.

1. Introduction

Cadmium (Cd) is a toxic heavy metal with a biological half-life of more than 20 years; its level in the environment is increasing due to industrial activities, thereby increasing human exposure to Cd [1,2]. It has been reported to bio-accumulate in many organs, including the liver, kidney, pancreas, and testis, and adversely affect the functions of

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these organs [3]. Among the various organs, the kidney is recognized as a major target of cadmium-induced renal toxicity due to its preferential uptake by receptor-mediated endocytosis and metallothionein-bound Cd in the renal proximal tubule [3]. When released freely into the cytosol, it can generate reactive oxygen species (ROS) and activate cell death pathways [3].

Epidemiological and experimental evidence suggested that acute Cd exposure induces oxidative stress through the inhibition of antioxidant enzymes, increased level of lipid peroxidation, and depletion of sulfhydryl (SH)-group-containing compounds [2,4]. However, the toxic effects of Cd are rather complex and still debated [5]. This has been the focus of much research, but there are more factors yet to be identified and explored.

Previous studies have highlighted the relationship of oxidative stress and nitric oxide (NO) production in kidney function under normal and pathological conditions [6–8]. Free radicals such as superoxide radical (O_2^-) can interact with NO forming peroxynitrite ($ONOO^-$), thereby depleting NO bioavailability.

NO is a potent, endogenous vasodilator that regulates renal function, among other functions [7]. It is produced from L-arginine by endothelial nitric oxide synthase (eNOS); however, arginase competes with this enzyme for the same substrate to produce urea and L-ornithine [9]. In the kidney, increased arginase activity can thus reduce availability of L-arginine for eNOS, causing a decrease in NO production and a rise in superoxide generation due to uncoupling of eNOS [9].

Studies have also implicated the endogenous signaling molecule adenosine in kidney function. Adenosine is produced by enzymatic phosphohydrolysis of its precursor molecules, particularly Adenosine triphosphate (ATP) and Adenosine monophosphate (AMP) [10–12]. However, adenosine deaminase (ADA), an enzyme, that is present in the kidney, catalyzes the irreversible hydrolytic deamination of adenosine to inosine and 2-deoxyadenosine to 2-deoxyinosine, thereby depleting the level of adenosine production. Therefore, inhibition of ADA activity has been suggested to be a good therapeutic approach for the management/prevention of kidney dysfunction.

Curcumin is the principal natural polyphenol curcuminoid of turmeric (*Curcuma longa*) rhizome, a member of the ginger family (Zingiberaceae) [13]. Curcumin has a wide spectrum of therapeutic properties, and it has been shown to possess antioxidant, anti-inflammatory, anticancer, antiangiogenesis, chemopreventive, and chemotherapeutic properties [14–18]. Studies on the effect of curcumin on enzymes involved in degradation of renal adenosine and L-arginine metal toxicity in animal models are scarce. Therefore, the present study highlighted other significant aspects that underline Cd/curcumin exposure. Hence, we investigated the effect of curcumin on arginase and ADA activities in Cd-induced renal toxicity in rats.

2. Materials and methods

2.1. Chemicals

Cadmium sulfate was obtained from Oxford Laboratory, Mumbai, India, and curcumin was purchased from Sigma-Aldrich, St Louis, MO, USA. All other reagents used in this study were of analytical grade, and water was glass distilled.

2.2. Animals and experimental design

Adult male albino rats (weighing 150–180 g) were obtained from the animal breeding unit at Afe Babalola University, Ado-Ekiti, Nigeria, and were housed in cages, at room temperature (25–28°C), relative humidity 60–70%, and 12-hour light/dark cycle. Food (pellet rat chow) and water were available *ad libitum*. Animals were cared according to US National Institute of Health ethical guidelines.

After 2 weeks of acclimatization, animals were randomly divided into six groups of six animals each: saline/vehicle, saline/curcumin 12.5 mg/kg, saline/curcumin 25 mg/kg, Cd/vehicle, Cd/curcumin 12.5 mg/kg, and Cd/curcumin 25 mg/kg. In the present study, Cd sulfate was administered orally to rats as described by Zalups and Ahmad [19], and the choice of Cd dosage was according to the study of Goncalves et al [20], where it induced renal damage, while the choice of the curcumin doses (12.5 mg/kg and 25 mg/kg) was made based on previous works that reported beneficial results of this compound in rats [21]. Both solutions were administered for a period of 7 days. Curcumin was administered 30 minutes after Cd, and the solutions were freshly prepared. Cd was diluted in saline and the curcumin in 0.1% ethanol, and both were administered (1 mL/kg).

It is important to note that controls for all *ex vivo* tests were performed to correct vehicle (0.1% ethanol) interference. However, no significant differences were observed between the results obtained for the vehicle (0.1% ethanol) and the control (saline) regarding the parameters analyzed in this study (data not shown).

After the treatment period, animals were fasted overnight and sacrificed 24 hours after the last dose under light ether anesthesia. Blood samples were obtained by heart puncture and centrifuged at 3000*g* for 10 minutes. The clear non-hemolyzed sera were stored at −20°C till subsequent measurements. The kidneys were quickly excised and washed in cold saline solution, blotted on filter papers to remove adhering blood, and homogenized in 100mM potassium phosphate, pH 7.5. The homogenates were centrifuged at 10,000*g* for 20 minutes at 4°C, and the supernatant was used for subsequent enzymatic assays.

2.3. Determination of ADA activity

ADA activity determination was performed as described by Guisti and Galanti [22], which is based on the direct measurement of the formation of ammonia, produced when ADA acts in an excess of adenosine. In brief, 50 μL of kidney homogenates reacted with 21 mmol/L of adenosine, pH 6.5, and was incubated at 37°C for 60 minutes. The protein content used for the experiment was adjusted to between 0.7 mg/mL and 0.9 mg/mL. The results obtained were expressed in units per liter (U/L). One unit (1 U) of ADA is defined as the amount of enzyme required to release 1 mmol of ammonia per minute from adenosine at standard assay conditions.