Available online at www.sciencedirect.com
ScienceDirect
Clinica Chimica Acta 386 (2007) 94–99
www.elsevier.com/locate/clinchim

Vitamin C attenuates potassium dichromate-induced nephrotoxicity and alterations in renal brush border membrane enzymes and phosphate transport in rats

Sabiha Fatima, Riaz Mahmood *

Department of Biochemistry, Faculty of Life Sciences, Aligarh Muslim University, Aligarh 202002, U.P., India

Received 10 April 2007; received in revised form 8 August 2007; accepted 16 August 2007
Available online 24 August 2007

Abstract

Background: Exposure to chromium compounds can result in nephrotoxicity. The administration of potassium dichromate ($K_2Cr_2O_7$), a hexavalent chromium compound, results in impairment in functions of renal brush border membrane (BBM).
Methods: The effect of vitamin C (ascorbic acid) on $K_2Cr_2O_7$-induced nephrotoxicity, changes in BBM enzymes, Pi transport and the anti-oxidant status of rat kidney were studied. Animals were divided into 4 groups and were intraperitoneally given saline (control), vitamin C alone, $K_2Cr_2O_7$ alone and vitamin C plus $K_2Cr_2O_7$. Nephrotoxicity was evaluated by urea and creatinine levels in the serum. Anti-oxidant status was evaluated in kidney homogenates.
Results: A single dose of $K_2Cr_2O_7$ (15 mg/kg body weight) resulted in an increase of serum urea nitrogen and creatinine levels, increase in lipid peroxidation and decrease in total sulfhydryl groups. However, prior treatment with a single dose of vitamin C (250 mg/kg body weight) protected the kidney from the damaging effects of $K_2Cr_2O_7$. It greatly ameliorated the $K_2Cr_2O_7$-induced nephrotoxicity and reduction in Pi transport, activities of catalase, Cu–Zn superoxide dismutase and BBM enzymes. This was accompanied by decrease in lipid peroxidation and recovery of sulfhydryl content of renal cortex.
Conclusions: Vitamin C is an effective chemoprotectant against $K_2Cr_2O_7$-induced acute renal failure and dysfunction of the renal BBM in rats.

Keywords: Potassium dichromate; Nephrotoxicity; Brush border membrane; Phosphate transport; Vitamin C; Oxidative stress

1. Introduction

Chromium (Cr) is widely used in industries with uses in steel, alloy cast irons, chrome plating, paints, metal finishes, leather tanning, photography, etc. [1]. Occupational exposure to Cr has been associated with welders, chrome plating and chromium pigment workers. Cr compounds are toxic, carcinogenic and mutagenic in humans and animals [2]. Dermal, renal and hepatic toxicities have been reported in Cr exposed humans. Cr exists in many valence states but the trivalent [Cr(III)] and hexavalent [Cr(VI)] forms are of biological importance and are also the most prevalent Cr compounds in the workplace. Cr(VI) compounds are the most toxic since they can be easily absorbed and transported across membranes via non-specific anion carriers [3,4]. The toxic effects of Cr are attributed to its ability to induce oxidative stress leading to enhanced production of reactive oxygen species (ROS). This results in a decreased cell viability, enhanced intracellular oxidized states, membrane damage and apoptotic and necrotic cell death [5].

The kidney is the main target organ for Cr accumulation and is thus more sensitive to the toxic effects of Cr than other tissues [6]. Administration of potassium dichromate ($K_2Cr_2O_7$), a Cr(VI)

Abbreviations: AP; alkaline phosphatase; ARF; acute renal failure; BBM; brush border membrane; BBMV; BBM vesicles; BUN; blood urea nitrogen; Cu–Zn SOD; copper–zinc superoxide dismutase; Cr; chromium; GGTase; gamma-glutamyl transferase; GSH; glutathione; HEPES; *N*-2-hydroxyethylpiperizine-*N*′-2-ethanesulfonic acid; $K_2Cr_2O_7$; potassium dichromate; LAP; leucine aminopeptidase; Mlt; maltase; MDA; malondialdehyde; Pi; inorganic phosphate; ROS; reactive oxygen species; Scr; serum creatinine; SH; sulfhydryl; Tris; tris(hydroxymethyl)aminomethane.

* Corresponding author. Tel.: +91 571 2700741.
E-mail address: riazmahmood2002@yahoo.co.in (R. Mahmood).

0009-8981/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.cca.2007.08.006

compound, is toxic to the kidney and leads to acute renal failure (ARF) that appears to be reversible [7,8]. In fact ARF induced by $K_2Cr_2O_7$ has been used as a model to study the disease. The convoluted section of the proximal tubules is most affected by exposure to $K_2Cr_2O_7$. Clinical and experimental renal damage induced by $K_2Cr_2O_7$ has also been associated with oxidative stress with ROS being implicated in inducing cell injury [8,9]. Free radical scavengers like vitamin E protect against $K_2Cr_2O_7$-induced changes which strongly suggests that the renal effects of Cr(VI) may be due to enhanced production of ROS [10].

The brush border membrane (BBM) lining the epithelial cells of the kidney is a major target of renal injury due to ischemia and nephrotoxic agents. We have previously shown that administration of a single dose of $K_2Cr_2O_7$ to rats resulted in ARF and a significant reduction in the activities of several renal BBM enzymes and Na^+-dependent inorganic phosphate (Pi) transport across BBM vesicles [11]. The ARF was reversible after 8 days with maximum damage being observed 48 h after the administration of $K_2Cr_2O_7$. It was suggested that this impairment in the functions of renal BBM may play an important role in mediating the toxic effects of $K_2Cr_2O_7$ in the kidney.

Extensive research has led to a better understanding of the biochemical mechanisms by which food components influence health and disease. Diet controls and modulates many important functions of the body and participates in the maintenance of good health or homeostasis to reduce the risks of many chronic disorders. Vitamin C (ascorbic acid) is an essential nutrient that functions as a reducing agent and non-enzymatic anti-oxidant in the cell [12]. The use of vitamin C in alleviating the renal biochemical changes induced by nephrotoxic doses of Cr(VI) has not been examined previously.

2. Materials and methods

2.1. Chemicals

L-leucine *p*-nitroanilide, γ-glutamyl *p*-nitroanilide, glucose oxidase, horse radish peroxidase, mannitol, tris(hydroxymethyl)aminomethane (Tris) and *N*-2-hydroxyethylpiperizine-*N*′-2-ethanesulfonic acid (HEPES) were from Sigma-Aldrich Chemical Co (St Louis, MO) while *p*-nitrophenyl phosphate and $K_2Cr_2O_7$ were from Sisco Research Laboratory (Mumbai, India). ^{32}P-labeled inorganic phosphate (^{32}Pi) was from Bhaba Atomic Research Centre (Mumbai, India). All other chemicals were of the highest purity available.

2.2. Animals and experimental design

Adult male Wistar rats weighing 180–200 g were used in the present studies. The animals were kept and utilized under humane conditions in compliance with the present institutional guidelines. All animals had free access to food and water and were acclimatized to laboratory conditions for 1 week before the experiments. The animals were randomly divided into 4 groups with 6 rats in each group. Animals in the first group received 1 ml of normal saline alone and served as the control. The second group ($K_2Cr_2O_7$ alone) was given a single intraperitoneal (i.p.) dose of $K_2Cr_2O_7$ in saline (15 mg/kg body weight) while the third group (vitamin C alone) received a single i.p. dose of vitamin C in saline (250 mg/kg body weight). Animals in the 4th group (vitamin C+ $K_2Cr_2O_7$) were given a single i.p. injection of vitamin C (250 mg/kg), 6 h before the administration of $K_2Cr_2O_7$ (15 mg/kg). The dose of $K_2Cr_2O_7$ (15 mg/kg body weight) used in this study has been used previously by other investigators since it is nephrotoxic but not lethal to the animals [7,8,11]. All animals survived the duration of the experiment. The vitamin C dose of 250 mg/ kg gave good protection from the nephrotoxic effects of $K_2Cr_2O_7$. Lower doses of vitamin C gave less protection while higher doses were not much more effective.

All animals were sacrificed 48 h after the above treatments under light ether anesthesia; their blood and kidneys were removed and used in further analyses. The animals were sacrificed 48 h after the administration of $K_2Cr_2O_7$ since our previous work has shown that renal damage is maximum at this time [11].

2.3. Preparation of BBM vesicles and homogenates

The kidneys were decapsulated and the whole cortex and medulla regions were carefully separated. The whole cortex tissue from each animal was homogenized separately in 2 mmol/l Tris–HCl, 50 mmol/l mannitol buffer, pH 7 to give a 10% (w/v) homogenate. After dilution to 5% with Tris–mannitol buffer aliquots of the homogenates were saved at −20 °C. BBM vesicles (BBMV) were prepared from the whole cortical homogenates using the $MgCl_2$ precipitation method as described previously [13]. The final membrane preparations were suspended in 5 mmol/l Tris-HEPES, 300 mmol/l mannitol buffer, pH 7.4, and used immediately or frozen at −20 °C until further use.

2.4. Enzyme assays

The activities of BBM enzymes were assayed in isolated BBMV and cortical homogenates. The activity of alkaline phosphatase (AP) was determined by following the hydrolysis of *p*-nitrophenyl phosphate at 410 nm [14]. Leucine aminopeptidase (LAP) and γ-glutamyl transferase (GGTase) were assayed using L-leucine *p*-nitroanilide and γ-glutamyl *p*-nitroanilide as substrates, respectively [15,16]. Maltase (Mlt) was assayed by the glucose oxidase–peroxidase method [17]. Kinetic parameters K_m (Michaelis constant) and V_{max} (maximum velocity) were determined by assaying the enzymes in isolated BBMV at different substrate concentrations. The substrate concentrations (in mmol/l) used in these kinetic studies were: AP-0.05 to 0.4; GGTase-0.13 to 1.0; Mlt-3.3 to 40; LAP-0.13 to 1.0.

Cu–Zn superoxide dismutase (Cu–Zn SOD) was assayed by following the inhibition of auto-oxidation of pyrogallol and catalase from the conversion of hydrogen peroxide to water at 240 nm [18,19]. Protein concentrations in the homogenates and BBMV were determined by the Folin phenol reagent using bovine serum albumin as the standard [20].

2.5. Malondialdehyde levels, sulfhydryl groups and serum parameters

Total sulfhydryl (SH) groups and malondialdehyde (MDA) levels were measured colorimetrically in the cortical homogenates after reaction with 5,5′-dithiobisnitrobenzoic acid and thiobarbituric acid, respectively [21,22]. Blood urea nitrogen (BUN) levels were measured in serum samples by the diacetyl monoxime method while serum creatinine (Scr) was determined in deproteinized serum after reaction with alkaline picrate solution [23,24].

2.6. Phosphate transport

The uptake of Pi across BBMV was measured by the rapid filtration technique [25]. The uptake was measured at 25 °C using ^{32}Pi in the presence (100 mmol/l NaCl) and absence (100 mmol/l KCl) of a Na^+-gradient.

2.7. Data analysis

All data are expressed as mean ± SE. Statistical evaluation was conducted by the group *t*-test. A $p < 0.05$ was used as the criterion for statistical significance. All experiments were done at least 3 times to document reproducibility.

3. Results

The effect of pre-treatment with vitamin C on $K_2Cr_2O_7$-induced nephrotoxicity and changes in BBM enzymes, phosphate transport and anti-oxidant status of the cell was studied. Rats were