Food Chemistry 134 (2012) 980–985

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Oral administration of a nephrotoxic dose of potassium bromate, a food additive, alters renal redox and metabolic status and inhibits brush border membrane enzymes in rats

Mir Kaisar Ahmad [a], Ashreeb Naqshbandi [a], Mohd Fareed [b], Riaz Mahmood [a,*]

[a] Department of Biochemistry, Faculty of Life Sciences, Aligarh Muslim University, Aligarh 202002, U.P., India
[b] Department of Zoology, Faculty of Life Sciences, Aligarh Muslim University, Aligarh 202002, U.P., India

ARTICLE INFO

Article history:
Received 27 October 2011
Received in revised form 30 January 2012
Accepted 1 March 2012
Available online 7 March 2012

Keywords:
Brush border membrane
Carbohydrate metabolism
Kidney
Oxidative stress
Potassium bromate

ABSTRACT

The time dependent effect of orally administered $KBrO_3$ on redox status and enzymes of brush border membrane (BBM) and carbohydrate metabolism has been studied in rat kidney. Animals were given a single oral dose of $KBrO_3$ (100 mg/kg body weight) and sacrificed at different times after this treatment; control animals were not given $KBrO_3$. The administration of $KBrO_3$ resulted in nephrotoxicity, a decline in the specific activities of several BBM marker enzymes and also induced oxidative stress in kidney. The specific activities of enzymes of carbohydrate metabolism were also altered and suggest a shift in energy metabolism from the aerobic to anaerobic mode. The renal effects of single oral dose of $KBrO_3$ appeared to be reversible; maximum changes in all the parameters were 48 h after administration of $KBrO_3$ after which recovery took place, in many cases almost to control values, after 168 h. These results suggest that the administration of a single nephrotoxic dose of $KBrO_3$ inhibits brush border membrane enzymes, induces oxidative stress and alters energy metabolism of the renal system in a reversible manner.

1. Introduction

Potassium bromate ($KBrO_3$) is extensively used in food and cosmetic industries (IARC, 1986). It is used in bakeries as a flour improver giving strength and elasticity to the dough during the baking process while also promoting the rise of bread. The resulting bread tends to be strong and spongy with fine crumb structure. Bromate also promotes gluten development in dough. $KBrO_3$ is used in treating barley in beer making, in cheese production and is commonly added to fish paste products in Japan. $KBrO_3$ is a component of permanent hair weaving solutions. Bromate is also a major disinfectant by-product generated from bromide containing raw waters that undergo ozonation and chlorination, and is frequently detected in tap-water and bottled water. Acute human exposure to $KBrO_3$ has occurred primarily by accidental or purposeful ingestion of $KBrO_3$ solutions from permanent hair-wave kits (IARC, 1986). Nephrotoxicity is the primary response to $KBrO_3$ intoxication with death occurring from solutions containing 12 g $KBrO_3$ (EPA, 2001). Acute exposure to bromate causes not only kidney failure but also neuropathological disorders such as vertigo, tinnitus and irreversible deafness (EPA, 2001). Chronic exposure to $KBrO_3$ causes renal cell carcinomas in rats, hamsters and mice and thyroid and mesothelioma tumours in rats (DeAngelo, George, Kilburn, Moore, & Wolf, 1998; Kurokawa, Maekawa, Takahashi, & Hayashi, 1990). Due to its cross species carcinogenicity, bromate is considered a probable human carcinogen and a complete carcinogen in animals.

Effective prevention of $KBrO_3$ toxicity by antioxidants (Farombi, Alabi, & Akuro, 2002; Nishioka, Fujii, Sun, & Aruoma, 2006) and induction of 8-hydroxy deoxyguanosine by $KBrO_3$ *in vitro* and *in vivo* strongly suggest a role for oxidative stress in $KBrO_3$ toxicity and carcinogenesis (Ballmaier & Epe, 1995; Murata et al., 2001; Umemura, Kitamura, Kanki, et al., 2004). A link between lipid peroxidation (LPO) and renal DNA damage has been observed (Sai, Takagi, Umemura, Hasegawa, & Kurokawa, 1991). Alterations in gene expression in the kidney, the target tissue, and oxidative modifications of proteins and lipids have been reported (Ahlborn et al., 2009; Khan & Sultana, 2005). Thus, generation of reactive oxygen species (ROS) and consequent oxidative modifications of biomolecules are thought to contribute to the toxicity of bromate.

The plasma membrane of epithelial cells lining the renal proximal tubule is composed of two morphologically and functionally distinct regions, the luminal brush border and the basolateral membranes. The brush border membrane (BBM) faces the lumen and is the first barrier for various solutes during absorption in the kidney. The BBM contains a number of hydrolytic enzymes and transport systems (Murer & Biber, 1993). Several studies have shown that the renal proximal tubule, and its BBM in particular, is a major target of renal injury due to ischemia and nephrotoxic

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

0308-8146/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2012.03.004

agents (Fatima, Arivarasu, Banday, Yusufi, & Mahmood, 2005; Scherberich, Wolf, & Schoeppe, 1993). The present work investigates the time dependent effects of a single oral dose of $KBrO_3$ on the activities of enzymes of carbohydrate metabolism and BBM and also parameters of oxidative stress. Although there are several reports on its nephrotoxicity, our work illustrates the effect of orally administered $KBrO_3$ on renal cortex and medulla separately. It was also reported for the first time the effect of $KBrO_3$ on the enzymes of carbohydrate metabolism and BBM. These studies are important since impairment in energy metabolism and activity of BBM enzymes can affect the reabsorptive properties of the kidney and lead to renal failure.

2. Materials and methods

2.1. Chemicals

Reduced and oxidized glutathione, thiobarbituric acid (TBA), reduced and oxidized nicotinamide adenine dinucleotide phosphate (NADPH and $NADP^+$), tris(hydroxymethyl)aminomethane (Tris), pyrogallol, hydrogen peroxide and 5,5′-dithiobisnitrobenzoic acid were purchased from Sisco Research Laboratories, India. L-leucine *p*-nitroanilide, *p*-nitrophenyl phosphate, γ-glutamyl *p*-nitroanilide, 1-chloro-2–4-dinitrobenzene and $KBrO_3$ were from Sigma–Aldrich, USA. All other chemicals were of analytical reagent grade.

2.2. Animal protocol

Adult male Wistar rats weighing 150–200 g were used in the experiments. Animals were used under humane conditions that prevented them from experiencing unnecessary pain and discomfort in accordance with institutional guidelines. The study was approved by an institutional ethical committee that monitors research involving animals. All animals were stabilized for 1 week prior to the experiment on standard pellet rat diet with free access to water. $KBrO_3$ in drinking water was given orally as a single dose of 100 mg/kg body weight and the animals were sacrificed 12, 24, 48, 96 and 168 h later after being anesthetized with anesthetic ether. Control animals did not receive any $KBrO_3$ but were given an equivalent volume of water by gavage and were sacrificed along with the treated groups. The kidneys were removed and used for the preparation BBM vesicles (BBMV) and cortical and medullary homogenates. There were 8 rats in each group and all animals had free access to food and water for the entire duration of the experiment.

2.3. Urea nitrogen and creatinine levels

Blood was collected by cardiac puncture and mixed with glucose–citric acid–trisodium citrate as anticoagulant. The blood was then centrifuged at 900×*g* for 10 min at 4 °C in a clinical centrifuge and the plasma so obtained was used for determining urea nitrogen and creatinine levels. Urea nitrogen was determined in plasma by the diacetyl monoxime method using a kit from Span Diagnostics, India. Creatinine levels were determined in deproteinized plasma samples by using saturated picric acid.

2.4. Preparation of brush border membranes and homogenates

The kidneys were decapsulated and kept in ice-cold 154 mM NaCl and 5 mM Tris–HEPES buffer, pH 7.5. The cortex and medulla were carefully separated using a sharp scalpel and homogenized separately in a glass Teflon homogenizor in 2 mM Tris–HCl, 50 mM mannitol buffer, pH 7.0, to get a 10% (w/v) homogenate. These homogenates were diluted to 5% with Tris–mannitol buffer followed by high speed homogenization (20,000 revolutions per min) in an Ultra Turrex Kunkel homogenizor. The cortical and medullary homogenates were divided into aliquots and quickly frozen until further analysis. The BBM vesicles (BBMV) were prepared from whole cortical homogenates using the $MgCl_2$ precipitation method exactly as described by Khundmiri, Asghar, Khan, and Yusufi (1997). The final membrane preparations were suspended in 300 mM mannitol, 5 mM Tris–HCl buffer, pH 7.5 and used immediately or kept frozen until further use. Protein concentrations in homogenates and BBMV were determined by the method of Lowry, Rosebrough, Farr, and Randall (1951).

2.5. Assay of BBM enzymes

The enzymes were assayed in cortical and medullary homogenates and also BBMV as described by Khundmiri et al. (1997). Briefly, the activity of alkaline phosphatase was determined at pH 10.5 using *p*-nitrophenyl phosphate as substrate; γ-glutamyl transferase and leucine aminopeptidase were assayed using γ-glutamyl *p*-nitroanilide and L-leucine *p*-nitroanilide as substrates, respectively, and maltase by the glucose oxidase–peroxidase method.

2.6. Malondialdehyde, carbonyl content, GSH, total SH and H_2O_2 levels

The parameters of oxidative stress were determined in cortical and medullary homogenates. Malondialdehyde (MDA) was measured as TBA reactive substances (Buege & Aust, 1978) and carbonyl content was determined after reaction with 2,4-dinitrophenyl hydrazine (Levine et al., 1990). Total sulfhydryl (SH) groups and reduced glutathione (GSH) were determined from the yellow colour produced after reaction with 5,5′-dithiobisnitrobenzoic acid (Sedlak & Lindsay, 1968). Hydrogen peroxide (H_2O_2) levels were determined by using xylenol orange as colour reagent in the presence of 100 mM sorbitol (Gay & Gebicki, 2000).

2.7. Enzymes of carbohydrate metabolism

The activities of enzymes involving oxidation of NADH or reduction of $NADP^+$ were determined spectrophotometrically at 340 nm. Lactate dehydrogenase, malate dehydrogenase, glucose 6-phosphate dehydrogenase, malic enzyme, glucose 6-phosphatase and fructose 1,6-bisphosphatase activities were assayed as described by Khundmiri, Asghar, Khan, Salim, and Yusufi (2004).

2.8. Statistical analysis

All data are expressed as mean ± SEM. Statistical evaluation was conducted by one-way ANOVA using Origin Software 6.1 (USA). A probability level of $P < 0.05$ was selected as indicating statistical significance. All experiments were done at least three times to document reproducibility and results of one representative experiment are shown.

3. Results

3.1. Urea nitrogen and creatinine levels

Significant changes in plasma creatinine and urea nitrogen levels were seen after $KBrO_3$ treatment. Both parameters were increased after 12 h and peaked at 48 h, compared to the control group (Table 1). Recovery took place subsequently and the magnitude of increase was less at 96 h. Further recovery took place at 168 h but neither creatinine nor urea nitrogen levels had reached control values even at this time. This shows the induction of