Available online at www.sciencedirect.com
SCIENCE DIRECT®
Regulatory Toxicology and Pharmacology 42 (2005) 77–82
Regulatory Toxicology and Pharmacology
www.elsevier.com/locate/yrtph

The prooxidant effect of sodium metabisulfite in rat liver and kidney

Oğuz Elmas [a], Mutay Aslan [a], Serkan Çağlar [a], Narin Derin [b], Aysel Agar [c], Yakup Alıcıgüzel [a,*], Piraye Yargıçoğlu [b]

[a] Department of Biochemistry, Akdeniz University Medical School, Antalya, Turkey
[b] Department of Biophysics, Akdeniz University Medical School, Antalya, Turkey
[c] Department of Physiology, Akdeniz University Medical School, Antalya, Turkey

Received 16 December 2004
Available online 2 March 2005

Abstract

Sodium metabisulfite ($Na_2S_2O_5$) is used as an antioxidant and antimicrobial agent in a variety of drugs and functions as a preservative in many food preparations. In addition to their antioxidant activity, sulfites oxidize to sulfite radicals (SO_3^-) initiating lipid peroxidation. This study was performed to elucidate the effect of subchronic $Na_2S_2O_5$ (520 mg/kg/day) ingestion on hepatic and renal antioxidant enzyme activities and lipid peroxidation in albino rats. The antioxidant effect of L-carnitine was also tested in rats treated with $Na_2S_2O_5$. Plasma uric acid levels were monitored in all rats included in the study. Malondialdehyde (MDA) levels significantly increased in $Na_2S_2O_5$ treated rats vs. controls, with kidney values of 2.21 ± 0.21 vs. 1.22 ± 0.35 and liver values of 79.85 ± 19.5 vs. 31.36 ± 5.0 nmol/mg protein, respectively. Selenium-glutathione peroxidase (GPx) activity was significantly increased in $Na_2S_2O_5$ treated rats vs. controls, with kidney values of 38.22 ± 2.21 vs. 8.09 ± 0.76 and liver values of 31.11 ± 6.37 vs. 11.70 ± 1.02 U/g protein, respectively. Sodium metabisulfite treatment increased plasma uric acid levels in rats that were included in the study. No protective effect of L-carnitine was observed against lipid peroxidation in both liver and kidneys of rats treated with $Na_2S_2O_5$. The presented data confirm the prooxidant activity of sulfites and suggest that increased GPx activity and plasma uric acid levels may partially reduce the observed renal and hepatocellular oxidative damage caused via the ingestion of sulfites.

Keywords: Sodium metabisulfite; Lipid peroxidation; Liver; Kidney; Glutathione peroxidase

1. Introduction

Humans are exposed to both endogenous and exogenous sulfites. Considerable amount of sulfites is generated in vivo by the catabolism of sulfur-containing amino acids, cysteine and methionine (Cooper, 1983). Exogenous sources of sulfites include foods and beverages, ambient air, and pharmaceutical products. Five sulfite salts including sodium metabisulfite ($Na_2S_2O_5$), potassium metabisulfite ($K_2S_2O_5$), sodium bisulfite ($NaHSO_3$), potassium sulfite (K_2SO_3), and sodium sulfite (Na_2SO_3) are commonly used as antioxidants in food preparations (Gunnison and Jacobsen, 1987). Sulfites are also added as a preservative to a variety of pharmaceutical products. There are several amino acid preparations, utilized for parenteral nutrition, that contain a high level of sulfite (Lakamp and Dobesh, 2000). Similarly, the generic form of propofol, a drug used by anesthesiologists, is reported to contain 25 mg/mL $Na_2S_2O_5$ (Langevin, 1999).

Once ingested, sulfite salts react with water leading to the generation of bisulfite (HSO_3^-), sulfite (SO_3^{2-}), and sulfur dioxide (SO_2) (Gunnison, 1981). Indeed, sodium metabisulfite, potassium metabisulfite, sodium bisulfite, potassium sulfite, and sodium sulfite have all been

* Corresponding author. Fax: +90 242 227 44 82.
E-mail address: yakup@akdeniz.edu.tr (Y. Alıcıgüzel).

0273-2300/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.yrtph.2005.01.010

termed as "sulfating agents" because they release SO_2. Thus, the amounts of ingested sulfites are expressed as sulfur dioxide equivalents (SDE). An evaluation by the Federation of American Societies for Experimental Biology (FASEB) estimates that 30–100 mg SO_2 has no observed adverse effects for humans (Nair and Elmore, 2003). The FASEB report is further supported by a Joint Expert Committee in the World Health Organization (WHO) which has established an acceptable daily intake (ADI) level of sulfites as 0.7 mg/kg body weight, expressed as sulfur dioxide (Nair and Elmore, 2003). With this in mind, it is important to note that the mean per capita of sulfite intake from food and beverages is estimated as 19 mg SDE per day (Gunnison and Jacobsen, 1987). This level is reported to be 163 mg SDE in the 99th percentile of the population (Gunnison and Jacobsen, 1987).

The antioxidant effects of sulfites are due to the formation of SO_3^{2-} (Simon, 1986). Sulfite is a reductant that can undergo one- or two-electron oxidation forming a sulfite radical ($SO_3^{-\bullet}$) and sulfate (SO_4^{2-}), respectively (Mottley and Mason, 1988). The sulfite radical can further react with molecular oxygen forming a sulfite peroxyl radical ($SO_3OO^{\cdot}$) and a sulfate radical ($SO_4^{-\bullet}$) (Mottley and Mason, 1988). These sulfur centered free radicals can react with lipids leading to lipid peroxidation (Southerland et al., 1982).

In normal individuals, the amount of sulfites present in serum is low with reported levels of 4–5 nmol/L (Ji et al., 1995). This is probably due to the presence of sulfite oxidase (Cabre et al., 1990), a mitochondrial enzyme that catalyzes the oxidation of SO_3^{2-} to SO_4^{2-}, which is excreted in the urine (Tejnorova, 1978). Hepatic oxidation of exogenous sulfite is diffusion limited (Gunnison and Palmes, 1976). That is to say the liver metabolizes a constant fraction of sulfite it receives, but a limited amount will pass through the organ and enter the systemic circulation (Gunnison and Jacobsen, 1987). Sulfites can also undergo a nonenzymatic reaction with disulfide bonds generating glutathione *S*-sulfonate (Gunnison and Benton, 1971), causing an increase in intracellular glutathione (GSH) levels (Keller and Menzel, 1989). *S*-Sulfonate can be detected at low concentrations in the urine of healthy individuals, but are excreted in large amounts in sulfite oxidase deficient patients (Taylor et al., 1986).

The aim of this study was to determine the effect of $Na_2S_2O_5$ ingestion on rat hepatic and renal antioxidant enzyme activities, and to examine the role of sulfites in tissue peroxidation. The antioxidant effect of L-carnitine was also tested in rats treated with $Na_2S_2O_5$. L-Carnitine is an amino acid that is reported to improve the turnover of fatty acids peroxidated by reactive oxygen species (Di Giacomo et al., 1993).

Data reported herein confirm the prooxidant activity of sulfites, and suggest that increased tissue activity of GPx and elevated levels of plasma uric acid can partially reduce the observed renal and hepatocellular oxidative damage caused via the ingestion of sulfites.

2. Materials and methods

2.1. Animals

All experimental protocols conducted on rats were performed in accordance with the standards established by the Institutional Animal Care and Use Committee at Akdeniz University Medical School. Male albino Wistar rats weighing 200–250 g were housed in stainless steel cages in groups of five rats per cage and given food and water ad libitum. Animals were maintained at 12 h light–dark cycles and a constant temperature of 23 ± 1 °C at all times. Rats were divided into four groups of 10 animals each: Group 1, control; Group 2, rats treated with $Na_2S_2O_5$; Group 3, rats treated with L-carnitine; Group 4, rats treated with $Na_2S_2O_5$ + L-carnitine. L-Carnitine (Sigma-Tau, Rome, Italy; 50 mg/kg/day) and sodium metabisulfite (Merck, Darmstadt, Germany; 520 mg/kg/day) was given by intragastric intubation for 3 weeks (Hui et al., 1989). With reference to the reported theoretical yield of 67.39% SO_2 from $Na_2S_2O_5$ (Atkinson et al., 1993; Nair and Elmore, 2003), the given dose of 520 mg $Na_2S_2O_5$/kg/day was equivalent to 350 mg SO_2.

2.2. Enzyme activity measurements

At the end of the 21-day treatment period, rats were anesthetized with diethyl ether and sacrificed by exsanguination via the abdominal aorta. Liver and kidney were dissected and kept in liquid nitrogen prior to measurement of enzyme activities and lipid peroxidation. All tissues were weighed and homogenized in ice-cold 50 mM sodium phosphate buffer at pH 7.4. Homogenates were centrifuged (40,000*g*, 30 min, 4 °C), and supernatants were stored at −80 °C. The activities of Cu/Zn-superoxide dismutase (SOD), and selenium-GPx were assayed by the methods of Misra and Fridovich (1972), and Paglia and Valentine (1967), respectively. Protein concentrations in all samples were measured spectrophotometrically by a Lowry assay (Lowry et al., 1951) with bovine serum albumin as a standard.

2.3. Measurement of lipid peroxidation

Obtained tissue supernatants were transferred to clean tubes and used for lipid peroxidation analysis. Levels of MDA, a product of lipid peroxidation, were measured using the thiobarbituric acid (TBA) fluorometric assay (Wasowicz et al., 1993) with 1,1,3,3-tetra-