Effect of Sodium Metabisulfite on Oxidative Stress and Lipid Peroxidation Biomarkers

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Abstract: Background: Sulfites are widely used as preservatives in the foods and pharmaceutical agents. It has been demonstrated that sulfites can react with a variety of cellular components and cause toxicity.

Objective: The present study was designed to investigate the effects of ingested sodium metabisulfite (SMB) on serum antioxidant status in rats.

Methods: Thirty-two male Wistar rats were randomly divided into control and treated groups. Treated groups received 10, 100, and 260 mg/kg body weight of SMB for 28 days. After 28 days, serum was assayed for measuring superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), catalase (CAT) activities, glutathion (GSH) level and lipid peroxidation.

Results: The results showed that the activities of GPx, GR, CAT and GSH levels were significantly decreased in 100 and 260 mg/kg SMB treated rats, while malondialdehyde (MDA) level was significantly increased in 260 mg/kg treated group when compared with the control group.

Conclusion: It is concluded that SMB administration as dose-dependent is associated with decreased serum antioxidant enzyme activities and increased lipid peroxidation.

Keywords: Antioxidants, inflammation, lipid peroxidation, preservatives, stress oxidative, sulfite.

1. INTRODUCTION

Sulfiting agents including sodium metabisulfite are extensively used as preservatives and antioxidants in foods and drugs and have several industrial uses [1]. In addition to ingested sulfite, endogenous sulfite is continuously produced during the normal metabolism of sulfur-containing amino acids within tissues [2] and generated by neutrophils spontaneously or in response to stimulation from the bacterial endotoxin lipopolysaccharide [3]. Despite the apparent safety of the sulfite additives, reports in the early 1980s indicated that sulfite exposure was associated with adverse reactions including dermatitis, urticaria, hypotension, abdominal pain and diarrhea to life-threatening anaphylactic reactions especially in the adult asthmatic population [1]. Sulfite exposure induces the generation and release of inflammatory mediators [4]. Sulfites can react with a variety of cellular components including lipids, proteins, and DNA and can cause toxicity [3]. Cytotoxic effect of sulfites has been attributed to the formation of sulfur- and oxygen-based free radicals by neutrophils [5]. The impairment in mitochondrial membrane integrity and decreased ATP production could be the other mechanism of sulfite toxicity [6]. Sulfites have been documented to alter the oxidant and antioxidant balance in rat erythrocyte [7]. Adebayo’s study showed that ingestion of sodium metabisulfite (520 mg/kg/day) caused an increase in the MDA level and SOD activity and a decrease in the CAT activity in the rat testes compared to the control [8]. Taken together, sulfite-induced cytotoxicity is accompanied by inflammatory response, an increase of ROS formation, lipid peroxidation and depletin of glutathione [4, 9]. Decreased antioxidants enzymes activities or overproduction of free radicals can cause oxidative stress. It is a major risk factor in the onset and progression of many chronic degenerative diseases in humans [10, 11].

It is estimated that the humans consume 180-200 mg sulfite from foods and beverages in a single day which is more than the acceptable daily intake level (0.7 mg/kg body weight) [12]. Since the effects of different levels of ingested sulfite on serum markers of oxidative stress have rarely been studied, in this report the effects of doses less than 520 mg/kg of SMB on serum antioxidant enzyme activities and lipid peroxidation will be presented.

2. MATERIALS AND METHODS

A total of 32 male Wistar rats weighing 220-250 g were obtained from animal house of Shiraz University of Medical
Sciences, Iran. Rats were maintained at 12 h light-dark cycles and temperature of 23°C ± 2°C with free access to food and water. The procedures followed were in accordance with the standards set forth in the eighth edition of Guide for the Care and Use of Laboratory Animals. Islamic Azad University Animal Care and Usage Committee approved all experimental protocols used in our work.

2.1. Drugs Preparation

SMB was obtained from Sigma (EC No: 231-673-0, CAS. No: 7681-57-4). SMB was dissolved in distilled water. With reference to the reported theoretical yield of 67.39% SO2 from Na2S2O5 [13], the given dose of 10, 100 and 260 mg SMB/kg/day was equivalent to 7, 67 and 175 mg SO2.

2.2. Experimental Design

To clarify the dose dependent effect of sulfite on serum oxidant and antioxidant status in the present study, the different doses of SMB were chosen on the basis of earlier studies [12]. Rats were randomly divided into four groups of 8 animals each: 1) control group, 2) S10 group, 3) S100 group, 4) S260 group, to which 10, 100 and 260 mg/kg body weight of SMB was administered orally using feeding needle once a day for 28 consecutive days, respectively, while the control group received distilled water for the same period, as previously described by our team [14, 15]. One day after the last administration, rats were anaesthetized using diethyl ether and blood samples were collected by cardiac puncture.

2.3. Biochemical Analysis

The level of serum MDA, as a biomarker of lipid peroxidation, was determined using thiobarbituric acid (TBA) method. MDA reacts with TBA and produces a pink colored complex which has the maximum absorbance at 532nm. The results were expressed as nmol/L [16]. Serum glutathione (GSH) contents with 5-5'-dithiobis [2-nitrobenzoic acid] (DTNB) was measured and followed by a standard Ellman’s method [17]. The absorbance of the reaction products was observed after 5 min at 412 nm. The enzyme activities of glutathione peroxidase (GPx) was measured by continuous monitoring of the regeneration of reduced glutathione (GSH) from oxidized glutathione (G-S-S-G) upon the action of GR and NADPH according to the method of Fecondo and Augusteyn [18]. The activity of GR was assayed using the method described by Carlberg and Mannervik [19] with minor modifications. CAT was assayed spectrophotometrically by monitoring the decomposition of H2O2 using the procedure of Aebi [20]. The activity of SOD was determined according to Winterbourn et al. and is based on the ability of superoxide dismutase to inhibit the reduction of nitro-blue tetrazolium (NBT) by superoxide [21].

2.4. Statistical Analysis

The results were analyzed by one-way ANOVA using the SPSS (version18.0) software followed by post-hoc Tukey test. Values were considered significantly different at p < 0.05. The data were presented as mean ± SEM.

3. RESULTS

3.1. Effect of SMB on Serum Antioxidant Enzymes and Glutathione Level

As shown in Table 1, there is a significant decrease in GPx, GR, catalase activities and GSH level in S100 and S260 groups compared to the control group. However, there was no significant change in the mean serum SOD activity of the treated groups when values were compared with the control group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (u/ml)</th>
<th>GPx (u/ml)</th>
<th>GR (u/ml)</th>
<th>CAT (u/ml)</th>
<th>GSH (µmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.40 ± 0.74</td>
<td>17.10 ± 1.00</td>
<td>3.41 ± 0.20</td>
<td>19.18 ± 1.92</td>
<td>0.76 ± 0.08</td>
</tr>
<tr>
<td>S10</td>
<td>6.60 ± 1.05</td>
<td>17.36 ± 0.95</td>
<td>3.25 ± 0.19</td>
<td>17.90 ± 2.15</td>
<td>0.75 ± 0.07</td>
</tr>
<tr>
<td>S100</td>
<td>8.61 ± 0.57</td>
<td>4.20 ± 0.21***</td>
<td>1.98 ± 0.04***</td>
<td>8.86 ± 1.96’</td>
<td>0.46 ± 0.05’</td>
</tr>
<tr>
<td>S260</td>
<td>5.28 ±1.02</td>
<td>1.76 ± 0.26***</td>
<td>1.46 ± 0.04***</td>
<td>8.10 ± 3.13’</td>
<td>0.36 ± 0.06’*</td>
</tr>
</tbody>
</table>

Each value indicates the mean ± SEM. S10; S100; S260, administration of 10,100 and 260 mg/kg/day sodium metabisulfite, respectively for 28 days. SOD: superoxide dismutase; GPx: glutathione peroxidase; GR: glutathione reductase; CAT: catalase; GSH: reduced glutathione. Significant differences compared with controls are indicated by asterisks (* P < 0.05, ** P < 0.01, *** P < 0.001).

3.2. Effect of SMB on Serum MDA Level

As shown in Fig. (1), the administration of 100 mg/kg SMB resulted in a slight and non-significant increase in the serum level of MDA, but a profound increase of MDA levels (83%) was observed in the treated rats with 260 mg SMB when compared to the control group (p < 0.001).

4. DISCUSSION

The current study was conducted to determine whether SMB at different doses (10,100, 260 mg/Kg) has adverse effects on serum oxidative stress biomarkers in male Wister rats. Our findings clearly showed that the oral administration of sodium metabisulfite for 28 days caused a dose-dependent alteration of oxidant-antioxidant status in serum as evidenced by enhanced MDA and decreased GSH level and also CAT, GPx, and GR activities.
Exposure to the same dose of SMB caused an increase in MDA level and SOD activity and a decrease in CAT activity in the rat testis whereas the level of GSH was not significantly affected [8]. In a recent study, it was shown that hippocampus SOD, CAT and GPx activities were significantly increased by sulfite treatment [30]. In contrast, sulfite treatment (70 mg/kg) via drinking water had no effect on the antioxidant/oxidant balance in the rat plasma [30].

Since MDA is an index of lipid peroxidation, its serum level estimates the extent of peroxidation. We observed that the MDA level was significantly higher in the S260 group compared to the control rats, indicating an increase in the process of lipid Peroxidation. Results are in agreement with Adebayo et al. [8] and Ercan et al. [31]. Increased serum MDA level and decreased serum antioxidant enzymes activities and GSH level observed in this study can raise concerns for potential effects of sulfites on humans. Given the presence of considerable evidence to support the role of increased plasma markers of oxidative stress in the pathogenesis of many major disorders including cancer, ischemic heart disease and cataract, changes in many aspects of lifestyle including dietary habits should be considered. The recommended changes contain increased consumption of fruit and vegetable and organic foods instead of processed, packaged and fast foods containing preservatives or other additives.

CONCLUSION

The results obtained from this present study show that long term consumption of SMB reduces serum antioxidant level in male rats leading to an increase in lipid peroxidation. These data are a warning for people with high consumption of heavily processed foods, containing preservatives, like "Western diet".

ETHICS APPROVAL AND CONSENT TO PARTICI-

All experimental protocols used in our work were approved by Islamic Azad University Animal care and Usage Committee, Iran.

HUMAN AND ANIMAL RIGHTS

No human were used in this study. The procedures followed were in accordance with the standards set forth in the eighth edition of Guide for the Care and Use of Laboratory Animals.

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.
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