SHORT COMMUNICATION

EFFECT OF SULFUR DIOXIDE INHALATION ON ERYTHROCYTE ANTIOXIDANT STATUS AND LIPID PEROXIDATION IN EXPERIMENTAL DIABETES

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SUMMARY - The effect of sulfur dioxide (SO₂) on red cell antioxidant status and lipid peroxidation was examined in this research. Forty healthy male albino rats, aged three months, were divided into four equal groups: Control (C), SO₂+C (CSO₂), diabetic (D) and SO₂+D (DSO₂). Experimental diabetes mellitus was induced by i.v injection of alloxan with a dose of 50 mg/kg body weight. Ten ppm SO₂ was administered to the animals of SO₂ exposed groups in an exposure chamber for one hr/day × 7 days/wk × 6wks while other groups were exposed to filtered air in the same condition. SO₂ exposure, while markedly decreasing Cu, Zn-Superoxide dismutase (Cu, Zn-SOD) activity, significantly increased glutathione peroxidase (GSH-Px), catalase (CAT), glutathione (GSH) and glutathione-s-transferase (GST) activities and TBARS values in CSO₂ and DSO₂ groups compared with their respective control groups. From these results, it could be concluded that adaptative changes occurred in antioxidant systems that may counteract the free radical effect of SO₂ in the experimental groups.

Key-words: SO₂, diabetes, antioxidant enzymes, TBARS, rat, erythrocyte.

RéSUMÉ - Effet de l’inhalation du dioxyde de soufre sur le statut antioxidant érythrocytaire et la peroxydation lipidique sur un modèle de diabète expérimental

Ce travail a porté sur l’effet du dioxyde de soufre (SO₂) sur le statut antioxidant du globule rouge et sur la peroxydation lipidique. Quarante rats albinos mâles, âgés de 3 mois, ont été répartis en 4 groupes égaux: contrôle (C), SO₂+C (CSO₂), diabétique (D), et SO₂+D (DSO₂). Un diabète expérimental était induit par injection IV d’alloxane à la dose de 50 mg/kg de poids. 10 ppm SO₂ était administré aux animaux des groupes exposés au SO₂ dans une chambre d’exposition pendant une heure par jour × 7 jours par semaine × 6 semaines, tandis que les autres groupes étaient exposés à de l’air filtré dans les mêmes conditions.

L’exposition au SO₂ a réduit de façon nette l’activité supéroxyde dismutase (Cu, Zn-SOD) tout en augmentant de façon significative les activités glutathion peroxydase (GSH-Px), catalase (CAT), glutathione (GSH) et glutation -S-transferase (GST) ainsi que les valeurs des TBARS dans les groupes CSO₂ et DSO₂, par rapport à leur contrôle respectif. A partir de ces résultats, on peut conclure que les changements adaptatifs survivent dans les systèmes antioxydants qui pourraient contrebalancer les effets radicaux du SO₂ dans les groupes expérimentaux.

Mots-clés : SO₂, diabète, enzymes antioxydantes, TBARS, rat, érythrocyte.


**MATERIAL AND METHODS**

**Preparation of animals**

Forty healthy Swiss male albino rats, aged three months, were equally divided into four groups, control (C), control + SO₂ (CSO₂), diabetic (D), diabetic + SO₂ (DSO₂) groups. Experimental diabetes mellitus was induced by i.v. injection (caudal vein) of alloxan monohydrate in a dose of 50 mg/kg body weight. Ten ppm SO₂ was administered to the animals of SO₂ groups in an exposure chamber (1 m³) for one hr (8.00-9.00 a.m.) × 7 days/wk × 6 weeks. Control groups were exposed to filtered air in the same chamber for the same period of time. Daily food and water consumption of every cage and weekly weight of each rat were recorded during the feeding period. At the end of the experimental period (six weeks), rats were deprived of food for 24 hr and then prepared for the experimental procedure under ether anesthesia.

**Biochemical analysis**

Heparinized blood samples were taken by cardiac puncture. Blood was centrifuged at 1500 × g for 10 min at 4°C to separate erythrocytes from plasma. Erythrocytes were washed three times with cold sodium chloride (0.15 M). Erythrocyte Cu, Zn-SOD activities were expressed as the rate constant (k) of a first order reaction per gram hemoglobin. GST enzyme activities were determined in accordance with the method of Habig et al. [11]. All enzymatic activities were expressed per gram of hemoglobin at either 30°C (Cu, Zn-SOD, CAT, GST) or 37°C (GSH-Px). Erythrocyte glutathione (GSH) concentration was assayed by the method of Fairbanks and Klee [12]. Hemoglobin concentration of erythrocyte was determined by cyanmethemoglobin method [12]. Thiobarbituric acid reactive substances (TBARS) levels were determined according to the method of Wasowicz et al. [13]. The amount of lipid peroxides was expressed as nmol malondialdehyde/gr hemoglobin using 1,1, 3,3-tetraethoxypropane as standard.

**Statistical analyses**

Analysis of variance (ANOVA) was performed on all parameters for the factors of groups. Post hoc comparisons of the means were carried out using the Tukey’s test. Significance levels were set at p < 0.05.
RESULTS

The mean initial and final body weight, daily food and water intake of rats of the four groups are summarized in Table I. Final daily food and water consumptions of the diabetic and SO₂ exposed groups were significantly increased with respect to the initial values and the control group. As expected, diabetic groups had lower mean body weights compared with either pretreatment or respective control body weights.

The means of glucose, total cholesterol, LDL, HDL, and VLDL cholesterol and triglyceride (TG) values of all groups are shown in Table II. TG, total cholesterol, LDL, HDL, and VLDL cholesterol values were significantly increased in the diabetic groups as compared with the control groups. SO₂ exposure led to a significant increase in glucose level of the diabetic and control groups and a decrease in TG level of the diabetic group. As shown in Table III, elevated TBARS values and reduced Cu, Zn-SOD, GSH-Px and GSH activities were found in the diabetic group as compared with the control group. SO₂ exposure, while markedly decreasing Cu, Zn-SOD activity, significantly increased GSH-Px, CAT, GSH, GST activities and TBARS values in CSO₂ group and GSH-Px, CAT, GST and TBARS values in the DSO₂ group in comparison with their respective control groups.

DISCUSSION

Food consumption and plasma glucose levels were significantly increased in CSO₂ and DSO₂ groups in response to SO₂ exposure. Significant increments of plasma glucose levels in the SO₂ exposed groups are also in accordance with the study of Lovati et al. [1] showing the decrements of plasma insulin levels following 10 ppm SO₂ exposure. Moreover SO₂ exposure significantly decreased plasma TG level in DSO₂ group, because triglyceride catabolism was enhanced for compensatory energy supply in accordance with impaired glucose transport in this group [1].

Table I. The means of initial and final body weights, daily food and water consumptions of studied groups.

<table>
<thead>
<tr>
<th></th>
<th>Body Weight (g)</th>
<th>Daily Food Consumption (g/100 g, bw/day)</th>
<th>Daily water consumption (ml/100 g, bw/day)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Initial</td>
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<tr>
<td>Control (C)</td>
<td></td>
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<tr>
<td>Control + SO₂ (CSO₂)</td>
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<td>Diabetes (D)</td>
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<tr>
<td>Diabetes + SO₂ (DSO₂)</td>
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# Final vs initial; * Groups vs control group; ** Diabetes vs Control + SO₂; *** Diabetes + SO₂ vs Control + SO₂; **** Diabetes + SO₂ vs Diabetes; bw: Body weight
Table III. Antioxidant status and TBARS values of studied groups.

<table>
<thead>
<tr>
<th></th>
<th>GSH-Px (U/g Hb)</th>
<th>CAT (k/g Hb)</th>
<th>Cu-Zn-SOD (U/g Hb)</th>
<th>GSH (mg/dl)</th>
<th>GST (uMol/min/g Hb)</th>
<th>TBARS (nmol/g Hb)</th>
</tr>
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<tbody>
<tr>
<td>Control (C)</td>
<td>16.58 ± 3.97</td>
<td>222.80 ± 31.40</td>
<td>5160.80 ± 496.24</td>
<td>2.28 ± 0.30</td>
<td>206.30 ± 31.37</td>
<td>9.40 ± 1.87</td>
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<td>C + SO2 (CSO2)</td>
<td>20.97 ± 2.94</td>
<td>249.60 ± 22.35</td>
<td>3075.40 ± 503.09</td>
<td>2.73 ± 0.50</td>
<td>326.60 ± 103.74</td>
<td>12.60 ± 2.30</td>
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<td>Diabetes (D)</td>
<td>264.81 ± 30.11</td>
<td>82.68 ± 18.17</td>
<td>36.01 ± 8.43</td>
<td>27.19 ± 6.88</td>
<td>25.13 ± 8.77</td>
<td>105.18 ± 23.21</td>
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<tr>
<td>Diabetes + SO2 (DSO2)</td>
<td>301.01 ± 37.61</td>
<td>83.12 ± 8.83</td>
<td>31.05 ± 7.61</td>
<td>26.01 ± 8.90</td>
<td>84.64 ± 10.32</td>
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</tbody>
</table>

* Groups vs control; ** Diabetes vs Control + SO2; *** Diabetes + SO2 vs Control + SO2; **** Diabetes + SO2 vs Diabetes
Many contradictory results have been reported in the alterations of GSH-Px, SOD, CAT, GSH activities of RBC in diabetes mellitus [4]. Our data indicating the decrements of red cell GSH-Px, Cu, Zn-SOD and GSH activities in diabetes mellitus, is similar to those of other authors [4, 5, 7, 14, 15]. From the results obtained, Cu, Zn-SOD of erythrocytes appears to be one of the most susceptible proteins to glycation. The inactivation of Cu, Zn-SOD by glycation may enhance the accumulation of oxylipids. Significant elevation of lipid peroxidation in the diabetic group is also in agreement with this previous result [15]. Additionally, since the increased sorbitol synthesis causes NADPH depletion in diabetes mellitus [15], GSH concentration was decreased, because reduction of oxidised form of glutathione requires NADPH as cofactor and glutathione reductase.

SO₂ inhalation increased erythrocytes TBARS levels in the control and diabetic groups as compared with their respective control groups. A lipid peroxidative effect of SO₂ has been shown in previous studies [3, 16]. Based on our findings, it is likely that inhaled SO₂ increases free radicals which may amplify lipid peroxidation in diabetes mellitus. On the other hand, our findings clearly showed that SOD activity in RBC of rats exposed to SO₂ was diminished. It is perhaps not surprising that SOD molecules, comprising cysteine residues at their active site, should be affected by the formation of bisulfite [16]. Consequently, it may be concluded that SO₂ exposure results in an exaggerated release of free radicals and a decrement of Cu, Zn-SOD activity which represent a potential risk for the complications of diabetes mellitus.

As shown in a previous study [17], SO₂ exposure causes the production of H₂O₂ and organic hydroperoxides at the end of various reactions. Consistent with this data and other previous findings [2, 16], GSH-Px, CAT and GSH activities were found to be increased following SO₂ exposure. This result may be explained by the primary adaptation to provide some protection from lipid peroxidative effect of SO₂. Moreover in the present study, GST activity was increased following SO₂ exposure which may be due to inactivation of SO₂ by GST or formation of organic hydroperoxides by SO₂ radicals [16].

In conclusion, our present study showed that i) adaptive changes may occur to counteract free radical effect of SO₂ that might be a defence response to protect red cells from oxidative damage. ii) the changed physiological function of erythrocytes associated with diabetic state may be a possible factor for the incremental complications favored by SO₂ exposure.

REFERENCES