SHORT COMMUNICATION

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

A. AGAR, V. KÜÇÜKATAY, P. YARGIÇOĞLU, S. BILMEN, S. GÜMÜSLÜ, G. YÜCEL

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 péroxydation 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 albins 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 superoxyde dismutase (Cu, Zn-SOD) tout en augmentant de façon significative les activités glutathion peroxydase (GSH-Px), catalase (CAT), glutathion (GSH) et glutathion -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 surviennent 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.

Sulfur dioxide (SO₂), ubiquitous air pollutant, is produced by the processing of sulfur containing fossils fuels and many industrial processes [1]. Previous reports had been mainly concerned with short term exposures of animals or man and usually at higher concentrations than those encountered in urban atmospheres. SO₂ exposures of 5 or 10 ppm were used to simulate high urban or industrial scenarios [1, 2]. These studies [1, 3] have shown that SO₂ causes the formation of free radicals in aqueous environments which induce several functionally important alterations in red blood cells (RBC) such as increased oxidative denaturation products of both hemoglobin and cell membrane lipids etc., because free radicals induced by SO₂ exposure disturb the balance between the oxidant threat and the antioxidant defence. This balance is extremely important for RBC to remain in an optimal functional state, since mammalian red blood cells are particularly susceptible to oxidative damage due to several factors mentioned in a previous study [4]. It is well known that there are many enzymes which scavenge free radicals in mammalian organisms such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT) and glutathione-s-transferase (GST) [5].

Recently, increasing evidence claimed that free radicals were involved in the pathogenesis of various diseases such as diabetes mellitus [6]. Diabetes mellitus is a complex disease associated with hyperglycemia which is thought to accelerate the development of diabetic complications. Among them which reflects pathophysiology of RBC are glycated hemoglobin, protein damages [7]. Additionally, activity of antioxidant enzymes is altered in diabetes as reported previously [4, 7]. Thus, altered antioxidant defences in diabetes mellitus render red cells more susceptible to attacks by oxygen free radicals. Since the diabetic erythrocyte is less protected from the oxidant agents, it has a reduced mean survival as evidenced by some authors [4]. Therefore, it is reasonable to expect that SO₂ inhalation is a statistically significant risk factor for the complications of diabetes mellitus. However, the relationship between SO₂ pollution and oxidative status of RBC in diabetes mellitus has not yet been investigated. Regarding this point, the aims of this research were,

- to determine the extent to which alterations in major red blood cell (RBC) antioxidant enzymes (CAT, GSH-Px, SOD, GST) and glutathione (GSH) occurred in SO₂ exposed rats.
- to examine the effect of SO₂ exposure on antioxidant status and lipid peroxidation of red blood cells (RBC) in diabetic rats and to evaluate if SO₂ exposure might potentiate diabetic alterations of these parameters.

**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 (C(SO₂)), diabetic (D), diabetic + SO₂ (D(SO₂)) 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)/day × 7 days/wk × 6 weeks. Model MRU 95/3-CD apparatus was used to monitor the concentration of SO₂ within the chamber. 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 assayed by the spectrophotometric indirect inhibition technique of Misra and Fridovich [8]. GSH-Px activity was measured in erythrocytes by the coupled method of Paglia and Valentine [9]. CAT activity was measured in erythrocytes by the method of Aebi [10]. The method is based on the decomposition of H₂O₂ by the decrease in absorbance at 240 nm. Measurements were made at 30 °C and the results 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 cyanomethemoglobin 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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<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Initial</td>
</tr>
<tr>
<td>Control (C)</td>
<td>244.86 ± 8.31</td>
<td>294.91 ± 17.48</td>
<td>5.72 ± 0.49</td>
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<td>9.49 ± 0.79</td>
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<td>#p &lt; 0.0001</td>
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<tr>
<td>Control + SO₂ (CSO₂)</td>
<td>242.91 ± 18.60</td>
<td>292.00 ± 17.65</td>
<td>5.78 ± 0.56</td>
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<td>9.62 ± 0.79</td>
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<td>#p &lt; 0.001</td>
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<td>*p &lt; 0.01</td>
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<tr>
<td>Diabetes (D)</td>
<td>245.78 ± 14.61</td>
<td>176.66 ± 18.62</td>
<td>5.57 ± 0.50</td>
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<td>9.48 ± 0.54</td>
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<td>#p &lt; 0.0001</td>
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<td>*p &lt; 0.0001</td>
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<td>**p &lt; 0.0001</td>
</tr>
<tr>
<td>Diabetes + SO₂ (DSO₂)</td>
<td>251.19 ± 15.46</td>
<td>150.50 ± 15.33</td>
<td>5.59 ± 0.42</td>
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<td>9.43 ± 0.43</td>
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<td>****p &lt; 0.01</td>
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</tbody>
</table>

# Final vs initial; * Groups vs control group; ** Diabetes vs Control + SO₂; *** Diabetes + SO₂ vs Conrol+ SO₂; **** Diabetes + SO₂ vs Diabetes; bw: Body weight
### Table II. Glucose, total cholesterol, HDL, LDL and VLDL cholesterol and triglyceride values of control and experimental groups. Values represent the mean ± SD.

<table>
<thead>
<tr>
<th></th>
<th>Glucose (mg/dL)</th>
<th>Total cholesterol (mg/dL)</th>
<th>HDL-cholesterol (mg/dL)</th>
<th>LDL-cholesterol (mg/dL)</th>
<th>VLDL-cholesterol (mg/dL)</th>
<th>Triglyceride (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (C)</td>
<td>134.11 ± 19.14</td>
<td>51.02 ± 11.12</td>
<td>26.01 ± 8.10</td>
<td>19.87 ± 11.33</td>
<td>18.16 ± 7.06</td>
<td>71.07 ± 18.16</td>
</tr>
<tr>
<td>Control + SO₂ (CSO₂)</td>
<td>186.64 ± 38.12</td>
<td>59.11 ± 12.87</td>
<td>27.31 ± 8.83</td>
<td>20.12 ± 11.76</td>
<td>18.73 ± 9.69</td>
<td>86.06 ± 16.33</td>
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<tr>
<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>
</tr>
<tr>
<td>Diabetes + SO₂ (DSO₂)</td>
<td>301.01 ± 37.61</td>
<td>83.12 ± 9.81</td>
<td>36.88 ± 7.11</td>
<td>31.05 ± 7.61</td>
<td>26.01 ± 8.90</td>
<td>84.64 ± 10.32</td>
</tr>
</tbody>
</table>

* Groups vs control; ** Diabetes vs Control + SO₂; *** Diabetes + SO₂ vs Control + SO₂; **** Diabetes + SO₂ vs Diabetes

### Table III. Antioxidant status and TBARS values of red blood cells 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 (µmol/min/g Hb)</th>
<th>TBARS (nmol/g Hb)</th>
</tr>
</thead>
<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>
</tr>
<tr>
<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>
</tr>
<tr>
<td>Diabetes (D)</td>
<td>12.37 ± 2.23</td>
<td>243.10 ± 23.85</td>
<td>2455.00 ± 512.77</td>
<td>1.90 ± 0.29</td>
<td>188.90 ± 32.63</td>
<td>13.27 ± 1.21</td>
</tr>
<tr>
<td>Diabetes + SO₂ (DSO₂)</td>
<td>16.71 ± 2.68</td>
<td>278.70 ± 31.43</td>
<td>2250.40 ± 549.20</td>
<td>2.16 ± 0.41</td>
<td>288.30 ± 45.68</td>
<td>16.46 ± 1.81</td>
</tr>
</tbody>
</table>

* Groups vs control; ** D vs CSO₂; *** DSO₂ + CSO₂; **** DSO₂ + vs D
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, are 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 oxyradicals. Significant
elevation of lipid peroxidation in the diabetic group is
also in agreement with this previous result [15]. Addi-
tionally, since the increased sorbitol synthesis causes
NADPH depletion in diabetes mellitus [15], GSH con-
centration was decreased, because reduction of oxidi-
sed form of glutathione requires NADPH as cofactor
and glutathione reductase.

SO₂ inhalation increased erythrocytes TBARS lev-
els 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 cyto-
teine 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 exagger-
ated 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 hydroper-
oxides 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) ad-
aptive 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 asso-
ciated with diabetic state may be a possible factor for
the incremental complications favored by SO₂ expo-
sure.

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