J Physiol Biochem (2010) 66:205–212
DOI 10.1007/s13105-010-0025-7

ORIGINAL PAPER

Effect of sulfite on antioxidant enzymes and lipid peroxidation in normal and sulfite oxidase-deficient rat erythrocytes

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Received: 17 December 2009 / Accepted: 7 June 2010 / Published online: 23 June 2010

Abstract Sulfite and related chemical such as sulfite salts and sulfur dioxide has been used as a preservative in food and drugs. This molecule has also been generated from the catabolism of sulfur-containing amino acids. Sulfite is a very reactive and potentially toxic molecule and has to be detoxified by the enzyme sulfite oxidase (SOX). The aim of this study was to investigate the effects of ingested sulfite on erythrocyte antioxidant status by measuring glucose-6-phosphate dehydrogenase (G-6-PD), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities and oxidant status by measuring thiobarbituric acid reactive substances (TBARS) in normal and SOX-deficient rats. Rats were assigned to four groups (n=10 rats/group) as follows; control (C), sulfite (CS), deficient (D), and deficient+sulfite (DS). SOX deficiency was established by feeding rats a low molybdenum diet and adding to their drinking water 200 ppm tungsten (W). Sulfite (25 mg/kg) was administered to the animals via their drinking water. At the end of 6 weeks, Erythrocyte G-6-PD, SOD, and GPx but not CAT activities were found to be significantly increased with and without sulfite treatment in SOX-deficient groups. Sulfite treatment alone was also significantly increased erythrocytes' SOD activity in CS group compared to control. TBARS levels were found to be significantly increased in CS and DS groups and decreased in D group. When SOX-deficient rats treated with sulfite, TBARS level was still higher than other groups. In conclusion, these results suggested that erythrocyte antioxidant capacity, a defense mechanism against the oxidative challenge, increased by endogenous and exogenous sulfite due to its oxidant nature. This increase was also observed in CS and DS groups but it was insufficient to prevent lipid peroxidation.

Keywords Sulfite · Antioxidants · Lipid peroxidation · Erythrocyte · Rat

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Introduction

Sulfiting agents including sulfur dioxide, and various 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 frequently used as food additives to delay undesirable changes in color and preventing bacterial growth and as antioxidants in food preparations [6, 20, 39]. It has been shown that sulfite ions are formed by sulfite salts and sulfur dioxide in body fluids in case of taking various foods and beverages that contain sulfiting agents [21]. Endogenous sulfite is also generated as a consequence of the body's normal processing of sulfur-containing amino acids [11, 26]. Either ingested or endogenously generated, sulfite is a toxic molecule and has to be detoxified. For this purpose, mammalian tissues contain sulfite oxidase (SOX, EC. 1.8.3.1) which is a molybdenum (Mo)-containing enzyme located in the intermembraneous space of the mitochondria of mammalian tissues. High activity is seen in liver kidney and heart tissues whereas brain spleen and testis exhibit very low activities [4]. SOX catalyze the oxidative detoxification of sulfite to sulfate by a two-electron oxidation. Thus, mammalian cells are protected from its toxic effects [8]. Although there is little information about the mechanism of sulfite toxicity on the cell, sulfur- and oxygen-centered free radical-induced cell damage might play an important role in sulfite toxicity [1, 36]. Concurrently, it was also shown that sulfite-mediated oxidative stress leads to a depletion of intracellular ATP or energy failure [35].

Studies using ^{35}S-labeled sulfite have shown that 70–90% ^{35}S is absorbed into the bloodstream from the intestine and is distributed to body tissues via the blood [18]. Therefore, erythrocytes meet a great quantity of sulfite and they do not have SOX enzyme because they lack mitochondria. For this reason, erythrocytes may be considered to be among the most susceptible cells to sulfite's effects.

Erythrocytes are exposed to oxygen radicals continuously generated via the autooxidation of hemoglobin (Hb-Fe^{2+} into Hb-Fe^{3+}) [15]. In addition, the erythrocytes contain relatively high levels of unsaturated fatty acids which are good substrates for peroxidation reactions [38]. Erythrocytes are protected by antioxidant enzymes from peroxidative damage. The most important antioxidant enzyme in erythrocytes is glucose-6-phosphate dehydrogenase (G-6-PD; EC 1.1.1.49) which catalyzes the initial step of the pentose phosphate pathway. The most important function of G-6-PD is the reduction of NADP to NADPH which is used for the reduction of oxidized glutathione (GSSG) to a reduced state (GSH) and for the reduction of mixed disulfides of glutathione and cellular proteins [27]. Copper, zinc-superoxide dismutase (Cu, Zn-SOD; EC 1.15.1.1), a metalloprotein, catalyzes the dismutation of superoxide anions (O_2^-) to hydrogen peroxide (H_2O_2) and oxygen. Catalase (CAT; EC 1.11.1.6) catalyzes the degradation of H_2O_2 to H_2O and O_2. Selenium-dependent glutathione peroxidase (Se-GSH-Px; EC 1.11.1.9) catalyzes the reduction of H_2O_2 to H_2O at the expense of reduced glutathione [5].

Because of significant differences among animal species in their SOX activity [40], we used a SOX-deficient rat model to evaluate sulfite effect on erythrocyte antioxidant status. Most notable is the difference between rat and man. It was shown that rat liver has about a 10-20-fold greater SOX activity than human liver [24]. For this reason, it has been suggested that SOX-deficient rats might be a more useful model for the prediction of sulfite metabolism and toxicity in humans due to man's reduced SOX activity [12, 23].

Sulfite has some well-known effects on erythrocytes, such as decreasing 2,3-diphosphoglycerate levels [17], activating Ca-ATPase [28] and increasing erythrocytes deformability [3]. However, there are no reports concerning ingested sulfite-related changes of biomarkers for antioxidant enzyme activities such as G-6-PD, Cu, Zn-SOD, CAT, Se-GSH-Px, and the role of sulfites on lipid peroxidation in erythrocytes of SOX normal and SOX-deficient rats. Thus, the objective of the present investigation was to measure the activities of antioxidant enzymes (G-6-PD, Cu, Zn-SOD, CAT, Se-GSH-Px) and a marker of lipid peroxidation (thiobarbituric acid-reactive substances; TBARS) in erythrocytes of SOX normal and SOX-deficient rats. The present study was conducted on both SOX-normal and SOX-deficient rats, which represent a model for studying sulfite toxicity.

Materials and methods

Animals and treatment

Forty adult male Wistar albino rats weighing 200–220 g were used in this study. Pamukkale University Animal

Care and Usage Committee approved all experimental protocols used in our work. Four experimental groups, each consisting of ten rats, were formed: control group (C), group treated with sulfite (S), SOX-deficient group (D), and the deficient group treated with sulfite (DS). The rats in C and CS groups were given a standard rat chow and tap water, ad libitum. Sulfite in the form of $Na_2S_2O_5$ (70 mg kg^{-1} day^{-1}) was given orally by adding to drinking water to the CS and DS groups for 6 weeks. SOX deficiency in rats was induced by the administration of a low Mo diet (AIN 76, Research Dyets, USA) with concurrent addition of 200 ppm tungsten as sodium tungstate (NaWO4) to the drinking water of the D and DS groups (Table 1). This regimen was started 3 weeks before the sulfite treatment and continued for the whole experimental period. All groups exhibited similar weight gains and survival with no signs of toxicity.

Preparation of the hemolysates

At the end of sixth week, the animals were killed by exsanguination under urethane anesthesia. Blood samples were drawn from the abdominal aorta into tubes containing heparin as anticlotting agent. After centrifugation at 3,000×*g* for 10 min at 4°C, the plasma and buffy coat were removed. Erythrocytes were washed three times with an ice-cold isotonic sodium chloride solution (1:10, *v*/*v*) and the obtained packed cells were resuspended in the washing solution to give a 50% suspension. Hemolysis of the washed cell suspension was achieved by mixing 1 vol of cells with 9 vol of cold distilled water. The hemolysates were stored at −80°C for the determination of enzymatic activities of G-6-PD, Cu, Zn-SOD, CAT, Se-GSH-Px, and the levels of TBARS and hemoglobin.

Hepatic SOX assay

Hepatic SOX activity was determined as described in a previous paper [9]. Briefly, a portion of frozen liver (500 mg) was weigh and homogenized for 1 min at full speed with a Polytron in 5 ml 50 mmol phosphate buffer (pH 7.4). The homogenate was centrifuged at 3,500×*g* for 10 min at 4°C. An aliquot of supernatant (900 μl) was mixed with 5% Triton X-100 and further diluted (1:10) with 50 mmol phosphate buffer (pH 7.4). This diluted mixture was used for measuring SOX activity at room temperature by monitoring the reduction in cytochrome c at 550 nm. The mixture was added to the cuvette containing 10 mmol Na_2SO_3, 0.2 mmol cytochrome c, Triton X-100 (5%), 100 mmol Tris–HCl (pH 8.5), and KCN (10 mmol in Tris–HCl) in a final volume of 2.5 ml. The slow nonenzymatic rate of reduction in cytochrome c was first recorded, and its rate was subtracted from the recorded total rate. One unit of SOX activity was defined as the amount of enzyme, which caused an absorbance change of 0.1/min under these conditions. The results were expressed as units per milligram protein.

Enzyme activity measurements

SOD activity in erythrocytes was determined by the method of Misra et al [30]. Briefly, hemolysates were added to reaction mixture (50 mmol Na_2CO_3/$NaHCO_3$ buffer, 0,075 mmol EDTA and 3.6 mmol epinephrine HCL) in a final volume of 1.1 ml and SOD activity was measured at 30°C and 480 nm. Cu,

Table 1 Daily diet regimen and SOX status in experimental groups of normal and sulfite oxidase-deficient rats

Group	Hepatic SOX Activity (unite/mg protein)	Diet	Drinking water suplementation
Control	6.1±2.1	Standard rat chow	None
Sulfite treated	5.9±1.2	Standard rat chow	Sulfite (25 mg/kg)
Sulfite deficient	0.30±0.07*	Low Mo diet	W (200 ppm)
Sulfite deficient+sulfite treated	0.19±0.06*	Low Mo diet	W (200 ppm)+sulfite (25 mg/kg)

Values are expressed as mean±SEM; $p<0.05$ was considered as significant

SOX sulfite oxidase, *C* control groups, *CS* groups treated with sulfite, *D* SOX-deficient groups, *DS* SOX-deficient groups treated with sulfite, *W* molybdenum diet and tungsten in drinking water

*$p<0.0001$, compared with C and CS groups, ANOVA used

Zn-SOD activity measurement method was based on the ability of SOD to inhibit the autooxidation of adrenalin to adrenochrome at alkaline pH. Cu, Zn-SOD activities were expressed as units/g of hemoglobin (one unit, U=the amount of the sample which inhibits the transformation of adrenaline into adrenochrome by 50%).

Eryhtrocyte G-6-PD activity was measured by the modified method of Zinkham [19] by observing the conversion of $NADP^+$ into NADPH after addition of glucose-6-phosphate. Briefly, 50 μl hemolysate was mixed with 850 μl of reaction mixture (2 mM NADP+, 0.1M $MgCl_2$, 6 mM glucose-6-phosphate and Tris-HCl buffer (1 M, pH 8)) and the absorbance at 340 nm was monitored for 10 min. G-6-PD activities were expressed as units/g of hemoglobin (one unit, U=1 μmol of NADPH formed/min).

CAT activity was measured according to the method of Aebi [2]. The principle of the assay is based on the determination of the rate constant (s^{-1}, k) of hydrogen peroxide (H_2O_2) decomposition by CAT. The rate constant was calculated from the following formula: $k(2.3/8t)(a/b)\log(A_1/A_2)$. In this formula, A_1 and A_2 are the absorbance values of H_2O_2 at t_1 (0th s) and t_2 (15th s), a is the dilution factor, b is the hemoglobin content of erythrocytes. CAT activities were expressed as k/g of hemoglobin (k: rate constant of the first order reaction).

Se-GSH-Px activity was determined by the coupled assay of Paglia and Valentine [34] using t-butyl hydroperoxide as substrate. The decrease in NADPH was recorded at 340 nm and the molar absorptivity of NADPH, 6.22×10^{-3} L mol^{-1} cm^{-1} was used to calculate the enzyme activity. Se-GSH-Px activity was expressed as units/g of hemoglobin (1 unit, U=1 μmol of NADPH transformed /min).

All enzymatic activities were expressed per gram of hemoglobin at either 30°C (G-6-PD, Cu, Zn-SOD, CAT, and GST) or 37°C (Se-GSH-Px). The results for hemoglobin content were determined by using the cyanomethemoglobin method [14].

Measurement of lipid peroxidation

The lipid peroxidation contents of erythrocytes were determined by malondialdehyde (MDA) production and assayed as TBARS by using the method of Stocks and Dormandy [37] and the results were expressed as nmol/gHb using 1,1,3,3-tetraethoxypropane as standard.

Statistical analysis

Data was analyzed with a commercially available statistics software package (SPSS12 for Windows v. 9.0, Chicago, USA). Distributions of the groups were analyzed with a one-sample Kolmogrov–Smirnov test. One-way ANOVA test was performed and post hoc multiple comparisons were carried out with LSD. The results are presented as means±SEM. P values less than 0.05 were regarded as statistically significant.

Results

Hepatic SOX activity

Table 1 shows hepatic SOX activity at the end of the experimental period. Hepatic SOX activity can be used as an indicator for SOX status of the animals. Our results clearly demonstrate that induction of SOX deficiency by maintaining on the low-Mo diet with W supplementation (200 ppm) was very effective. Hepatic SOX activity in SOX-deficient groups was significantly reduced to about 1-5% of SOX normal groups ($p<0.0001$). Sulfite administration did not cause an alteration in SOX activity in either SOX normal or SOX-deficient animals.

Activities of antioxidant enzymes and the levels TBARS in erythrocytes

Effect of sulfite on G-6-PD, Cu, Zn-SOD, and Se-GSH-Px activities in normal and SOX-deficient rat erytrocytes is shown in Fig. 1. We observed a significant increase in G-6-PD activities in D and DS groups with respect to control and CS groups ($p<0.001$). Cu, Zn-SOD activities of all experimental groups are found to be significantly higher than that of the SOX normal control group (Fig. 1, $p<0.001$). A significant increase in Cu, Zn-SOD activity was observed in DS group compared with other groups ($p<0.001$). As shown in Fig. 1, the activities of Se-GSH-Px were significantly higher in D and DS groups than the control and CS groups ($p<0.001$ and $p<0.01$, respectively). The activities of CAT (mean± SEM; 126±12 IU/gHb, $P>0.05$) did not differ significantly among the groups.

TBARS levels are represented in Fig. 2. TBARS levels significantly increased in CS groups with

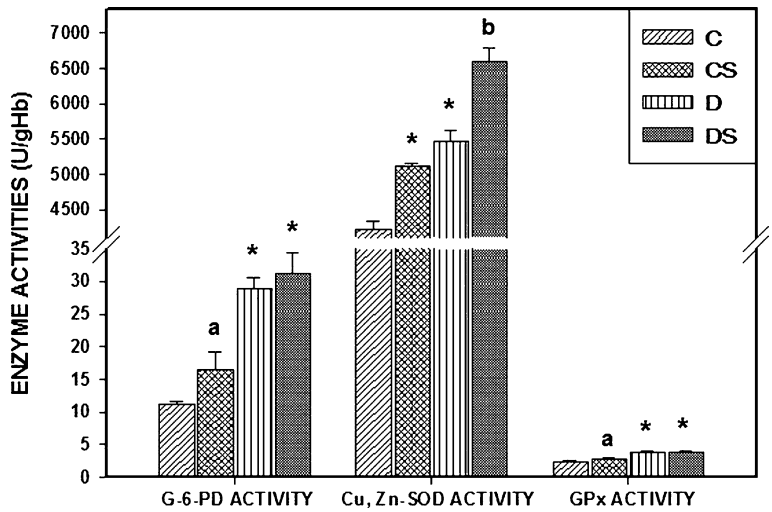

Fig. 1 Effect of sulfite on G-6-PD; Cu, Zn-SOD and GPx activity of erythrocytes in SOX normal and deficient rats (means±SEM; $n=10$. *C* control groups; *CS* groups treated with sulfite; *D* SOX-deficient groups; *DS* SOX-deficient groups treated with sulfite; *G-6-PD* glucose-6-phosphate dehydrogenase; *Cu, Zn-SOD* copper, zinc-superoxide dismutase; *GPx* glutathione peroxidase, and *CAT* catalase. *$p<0.001$ compared with control group; *a* $p<0.01$ compared with D and DS groups, *b* $p<0.001$ compared with the other groups ANOVA used)

respect to control group ($p<0.01$). Additionally, TBARS concentrations were significantly higher in DS group than all the other groups whereas it significantly decreased in D group compared to all other groups ($p<0.001$).

Discussion

Results of this study show that sulfite has stimulating effect on various erythrocyte antioxidant enzymes such as SOD, G-6-PD, and GPx in SOX-deficient groups. This effect was prominent especially in SOX-deficient rat's erythrocytes. Sulfite treatment alone was also significantly increased erythrocytes' SOD activity in CS group compared to control. According to the liver SOX activity measurements, SOX deficiency in rats was effectively induced with low-Mo diet with tungsten supplementation in the experimental groups of D and DS.

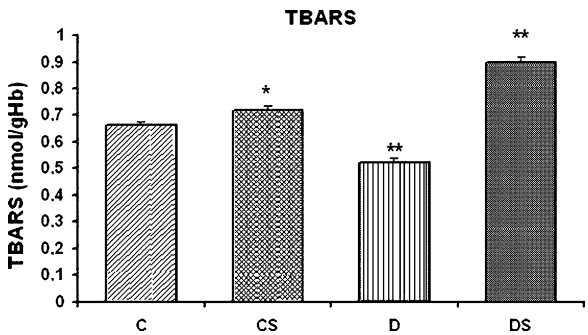

Fig. 2 Effect of sulfite on TBARS levels of erythrocytes in SOX normal and deficient rats (means±SEM; $n=10$. *C* control groups; *CS* groups treated with sulfite; *D* SOX-deficient groups; *DS* SOX-deficient groups treated with sulfite; and *TBARS* thiobarbituric acid reactive substances. *$p<0.05$ compared with control group, **$p<0.001$ compared with others groups, ANOVA used)

Sulfite compounds have been listed as "Generally Recognized as Safe" for use in foods and drugs to prevent enzymatic and nonenzymatic browning, control growth of microorganisms, act as bleaching agents and reducing agents, and carry out various other technical functions [39, 41]. The acceptable daily intake (ADI) for exogenous sulfite was established as 0–0.7 mg/kg body weight, expressed as sulfur dioxide [32] by The Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives. However, the daily intake of sulfite may be higher than ADI value. Indeed, studies have shown that it is possible to consume 180–200 mg/body weight from foods and beverages in a single day or meal that means there exist a potential for high consumers of sulfite to exceed the ADI [39]. For this reason, higher level of sulfite (25 mg/kg) was chosen in this study to observe possible adverse effects of it when highly consumed. The oral exposure route in drinking water was chosen

so as to mimic human exposure of sulfite. In addition to these exogenous sources of sulfite, it is also endogenously generated from the catabolism of sulfur-containing amino acids in the body. It is known that there are toxic effects of exogenous or endogenous sulfite on many cellular components [29, 33, 42]. Regardless of their source, sulfite should be metabolized to sulfate by SOX to protect the cell from its damaging effect.

In this study, G-6-PD activity increased in D and DS groups with respect to control and CS groups (Fig. 1). The increased G-6-PD activity in these groups may be a physiological response to sulfite-dependent oxidative stress. Studies indicated that the formation of GSH from its oxidized form, GSSG, is dependent on NADPH produced by the pentose phosphate pathway and that this pathway can be activated in response to GSH depletion [7]. Hence, the increased G-6-PD activity in these groups may be a physiological response to sulfite-dependent glutathione depletion in erythrocytes.

As shown in Fig. 1, increased erythrocyte SOD activities may be related to either ingested or endogenously generated sulfite-induced superoxide formation. The increment in SOD activity was significantly higher in DS group than other groups. Chemical studies of sulfite in the presence of oxygen have also revealed the generation of superoxide anion [31]. This formation triggers increased H_2O_2 production leading to protection from reactive oxygen species. This significant increment of SOD activity in all treatment groups may be an adaptive response to sulfite-dependent oxidative stress.

The H_2O_2 formed from SOD is removed by GPx, which catalyzes the reduction of H_2O_2 by GSH [10]. In this context, the observed increase GPx activities (Fig. 1) in erythrocytes of SOX-deficient and sulfite-exposed deficient rats can likely be due to increased substrate of the enzyme, H_2O_2. CAT catalyzes the reduction of H_2O_2 to H_2O and O_2 such as GPx [5]. According to our results, no significant difference was observed in the erythrocyte CAT activity among the different experimental groups. CAT activity of this invariability may depend on sulfite-induced superoxide anion radicals inactivating CAT activity.

Our finding that TBARS levels, an end product of lipid peroxidation, increased (Fig. 2) in the erythrocytes of sulfite-exposed normal (CS) and deficient groups (DS) suggesting the presence of increased oxidative stress. These results confirm previous observations of sulfite-induced lipid oxidation and further demonstrate its in vivo occurrence [13]. It is interesting to note that the TBARS levels in the erythrocytes of deficient groups (D group) were decreased compared to other groups. According to our results, sulfite treatment may cause oxidative stress and increases activity of antioxidant enzymes in all treatment groups compared to control. Under normal conditions, a delicate balance exists between rates of free radical formation and their removal by antioxidant enzymes [16]. Although an increment of one of the antioxidant enzymes activities may have a protective role, this is only true when adequate activity of the others exists [16]. In agreement with our results, there are a lot of studies which reports enhancement of cell tolerance to oxidative stress by modifying the cell antioxidant capacity [22, 25]. Therefore, the elevated activity of erythrocyte SOD, G6PD, and GPx in SOX-deficient groups (D and DS groups) may be related to sulfite based oxidative stress in present study. Although TBARS level was slightly elevated as an indicator of oxidant stress in SOX normal rats treated with sulfite, SOD activity also increased in the same group. Therefore, it can be speculated that rats can protect themselves against toxic effects of sulfite molecule due to their sufficient oxidative/antioxidative balance.

We have seen in our study that while SOX-deficient rats cope with this stressful condition with the help of the balance in their oxidative/antioxidative status, SOX-deficient and sulfite-treated rats (DS group) could not because of increased oxidant load. As seen in our results, erythrocyte antioxidant enzyme activities such as G6PD, GPx, and SOD increased in D and DS groups with respect to control group and SOD activity increased in DS group compared with other groups. However, TBARS level was significantly higher in DS group compared to other groups and higher in CS group compared to control. This increase indicates that elevated erythrocyte antioxidant capacity triggered by endogenous and exogenous sulfite mediated oxidative stress was insufficient to prevent lipid peroxidation in these groups. In addition, TBARS level was significantly lower in D group compared to other groups. Based on these observations, we could propose that exogenous sulfite causes more profound oxidative stress than endogenous sulfite.

In summary, these results obtained from rats with normal SOX activity could be due to the efficiency

whereby the SOX enzyme catalyses oxidation of sulfite to sulfate. Indeed, previous studies have shown that rat liver possessed approximately 10 to 20 times higher hepatic SOX activity than human liver. Because of both endogenous and exogenous sulfite overload, SOX-deficient rats which are an exaggerated model for the normal human situation cannot overcome this possible sulfite-dependent oxidative stress. Further studies are required to clarify these aspects.

References

1. Abedinzadeh Z (2001) Sulfur-centered reactive intermediates derived from the oxidation of sulfur compounds of biological interest. Can J Physiol Pharmacol 79:166–170
2. Aebi H, Cantz M, Suter H (1965) Cellular distribution of catalase activity in red cells of homozygous and heterozygous cases of acatalasia. Experientia 21:713–714
3. Bor-Kucukatay M, Kucukatay V, Agar A et al (2005) Effect of sulfite on red blood cell deformability ex vivo and in normal and sulfite oxidase-deficient rats in vivo. Arch Toxicol 79:542–546
4. Cabré F, Marín C, Cascante M, Canela EI (1990) Occurrence and comparison of sulfite oxidase activity in mammalian tissues. Biochem Med Metab Biol 43(2):159–162
5. Ceballos-Picot I, Trivier JM, Nicole A et al (1992) Age-correlated modifications of copper-zinc superoxide dismutase and glutathione-related enzyme activities in human erythrocytes. Clin Chem 38:66–70
6. Chapman K (1993) Sulfite-containing pharmaceuticals. Cmaj 148:714
7. Clancy RM, Levartovsky D, Leszczynska-Piziak J et al (1994) Nitric oxide reacts with intracellular glutathione and activates the hexose monophosphate shunt in human neutrophils: evidence for S-nitrosoglutathione as a bioactive intermediary. Proc Natl Acad Sci U S A 91:3680–3684
8. Cohen HJ, Fridovich I (1971) Hepatic sulfite oxidase. The nature and function of the heme prosthetic groups. J Biol Chem 246:367–373
9. Cohen HJ, Fridovich I, Rajagopalan KV (1971) Hepatic sulfite oxidase. A functional role for molybdenum. J Biol Chem 246:374–382
10. Cohen G, Hochstein P (1963) Glutathione peroxidase: the primary agent for the elimination of hydrogen peroxide in erythrocytes. Biochemistry 2:1420–1428
11. Dalton-Bunnow MF (1985) Review of sulfite sensitivity. Am J Hosp Pharm 42:2220–2226
12. Dulak L, Chiang G, Gunnison AF (1984) A sulphite oxidase-deficient rat model: reproductive toxicology of sulphite in the female. Food Chem Toxicol 22:599–607
13. Elmas O, Aslan M, Caglar S et al (2005) The prooxidant effect of sodium metabisulfite in rat liver and kidney. Regul Toxicol Pharmacol 42:77–82
14. Fairbanks VF, Klee GG (1999) Tietz textbook of clinical chemistry. In: Burtis CA, Ashwood ER (eds) Biochemical aspects of hematology. WB Saunders Company, Philadelphia, pp 1642–1710
15. Faivre B, Menu P, Labrude P et al (1998) Hemoglobin autooxidation/oxidation mechanisms and methemoglobin prevention or reduction processes in the bloodstream. Literature review and outline of autooxidation reaction. Artif Cells Blood Substit Immobil Biotechnol 26:17–26
16. Freeman BA, Crapo JD (1982) Biology of disease: free radicals and tissue injury. Lab Invest 47:412–426
17. Gerlach E, Duhm J, Deuticke B (1970) Metabolism of 2-3 diphosphoglycerate in red blood cells under various experimental conditions. In: Brewer GJ (ed) Red cell metabolism and function. Plenum Press, New York, pp 155–174
18. Gibson WB, Strong FM (1973) Metabolism and elimination of sulphite by rats, mice and nonkeys. Food Cosmet Toxicol 11:185–198
19. Group WS (1967) Standardization of procedures for the study of glucose-6-phosphate dehydrogenase. Report of a WHO Scientific Group. World Health Organ Tech Rep Ser 366:1–53
20. Gunnison AF, Jacobsen DW (1987) Sulfite hypersensitivity. A critical review. CRC Crit Rev Toxicol 17:185–214
21. Gunnison AF, Palmes ED (1973) Persistence of plasma S-sulfonates following exposure of rabbits to sulfite and sulfur dioxide. Toxicol Appl Pharmacol 24:266–278
22. Ji LL (1995) Exercise and oxidative stress: role of the cellular antioxidant systems. Exerc Sport Sci Rev 23:135–166
23. Johnson JL, Cohen HJ, Rajagopalan KV (1974) Molecular basis of the biological function of molybdenum. Molybdenum-free sulfite oxidase from livers of tungsten-treated rats. J Biol Chem 249:5046–5055
24. Johnson JL, Rajagopalan KV (1976) Human sulfite oxidase deficiency. Characterization of the molecular defect in a multicomponent system. J Clin Invest 58:551–556
25. Lee MY (2002) Effects of Na(2)SO(3) on the activities of antioxidant enzymes in geranium seedlings. Phytochemistry 59:493–499
26. Lester MR (1995) Sulfite sensitivity: significance in human health. J Am Coll Nutr 14:229–232
27. Lord-Fontaine S, Averill-Bates DA (2002) Heat shock inactivates cellular antioxidant defenses against hydrogen peroxide: protection by glucose. Free Radic Biol Med 32:752–765
28. Meltzer HL, Kassir S (1981) Enhanced activation of human erythrocyte Ca2± ATPase by calmodulin after storage or brief exposure to disulfite. Biochim Biophys Acta 643:243–250
29. Meng Z, Qin G, Zhang B et al (2004) DNA damaging effects of sulfur dioxide derivatives in cells from various organs of mice. Mutagenesis 19:465–468
30. Misra HP, Fridovich I (1972) The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 247:3170–3175
31. Mottley C, Mason RP (1988) Sulfate anion free radical formation by the peroxidation of (Bi)sulfite and its reaction with hydroxyl radical scavengers. Arch Biochem Biophys 267:681–689
32. Nair B, Elmore AR (2003) Final report on the safety assessment of sodium sulfite, potassium sulfite, ammonium

sulfite, sodium bisulfite, ammonium bisulfite, sodium metabisulfite and potassium metabisulfite. Int J Toxicol 22(Suppl 2):63–88
33. Ozturk OH, Kucukatay V, Yonden Z et al (2006) Expressions of N-methyl-D-aspartate receptors NR2A and NR2B subunit proteins in normal and sulfite-oxidase deficient rat's hippocampus: effect of exogenous sulfite ingestion. Arch Toxicol 80:671–679
34. Paglia DE, Valentine WN (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 70:158–169
35. Salman MS, Ackerley C, Senger C et al (2002) New insights into the neuropathogenesis of molybdenum cofactor deficiency. Can J Neurol Sci 29:91–96
36. Shi X (1994) Generation of SO3.- and OH radicals in SO3 (2-) reactions with inorganic environmental pollutants and its implications to SO3(2-) toxicity. J Inorg Biochem 56:155–165
37. Stocks J, Dormandy TL (1971) The autoxidation of human red cell lipids induced by hydrogen peroxide. Br J Haematol 20:95–111
38. Sukalski KA, LaBerge TP, Johnson WT (1997) In vivo oxidative modification of erythrocyte membrane proteins in copper deficiency. Free Radic Biol Med 22:835–842
39. Taylor SL, Higley NA, Bush RK (1986) Sulfites in foods: uses, analytical methods, residues, fate, exposure assessment, metabolism, toxicity, and hypersensitivity. Adv Food Res 30:1–76
40. Tejnorova I (1978) Sulfite oxidase activity in liver and kidney tissue in five laboratory animal species. Toxicol Appl Pharmacol 44:251–256
41. Wedzicha BL (1992) Chemistry of sulphiting agents in food. Food Addit Contam 9:449–459
42. Yi H, Liu J, Zheng K (2005) Effect of sulfur dioxide hydrates on cell cycle, sister chromatid exchange, and micronuclei in barley. Ecotoxicol Environ Saf 62:421–426