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Effects of Sulfur Dioxide Inhalation on Plasma Vitamin C and Ceruloplasmin in Ageing Rats

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Abstract: Ageing is associated with changes in physical characteristics and decline of many physiological functions. It has been accepted that the oxidative stress or damage induced by free radicals is related to ageing. Three age groups, 3, 12 and 24 months, were used to investigate whether age-associated changes in some parameters (vitamin C and ceruloplasmin) in the plasma of male Swiss-Albino rats and to observe possible effects of sulfur dioxide (SO_2) for 6 weeks on the same parameters. Rats were exposed to 10 ppm SO_2 1 hr/day, 7 days/week for 6 weeks. Control groups were exposed to filtered air in the same conditions. An effect of SO_2 on those parameters was observed. The level of vitamin C and ceruloplasmin in plasma were decreased in young, middle-aged and old groups in responce to SO_2.

Key words: Ageing, SO_2, Vitamin C, Ceruloplasmin, Rat

Many physical and biochemical changes take place during the process of ageing. Several theories have been proposed to explain ageing processes. Of these the free radicals theory of ageing is considered as a most reliable basis to ascertain the process of ageing because the free radicals are involved in decreasing the defense system and in causing damage to the cell structure and function[1)]. There are, however, defensive antioxidants like vitamin C (ascorbic acid) and ceruloplasmin (Cp) which detoxify these oxidants to protect cells.

Air pollution has a wide variety of effects on biological systems. Sulfur dioxide (SO_2) is one of the most common air pollutants of urban air[2)]. Studies using $^{35}SO_2$ have shown that inhaled SO_2 is readily distributed throughout the body[3)]. From the respiratory tract, SO_2 enters the blood[4)]. A free radical form of SO_2 exists in aqueous environments. This free radical ($SO_2^{-}\cdot$) is produced in aqueous SO_2 solutions by the action of light, chemical-reducing agents or by biochemical-reducing agents. The $SO_2^{-}\cdot$ radical metabolite can react with oxygen. The air oxidation of this free radical can form superoxide[5)].

Reactive oxygen species and other forms of free radicals must be rapidly neutralized by physiological defenses before they cause cellular dysfunction[6)]. The most important plasma antioxidants appear to be vitamin C and Cp[7–9)]. Vitamin C offers the most effective protection against plasma lipid peroxidation[10)] and it cooperates with vitamin E in the protection of cell membranes[11)]. Cp is considered to be a preventive plasma antioxidant because it can sequester transition metals, thereby preventing them from participating in free radical reactions[8, 10)].

This study was initiated to investigate the effect of SO_2 on the concentrations of some preventive antioxidants (vitamin C and Cp) in the plasma of male rats.

Seventy-eight healthy Swiss-Albino Male Albino rats (220–425 g) were used in this study. Animals were housed in a group of 4–5 rats in stainless steel cages at standard conditions (24 ± 2°C and 50 ± 5% humidity) with 12 hour light-dark cycle. They were separated into three equal groups according to age; young group (Y: 3 months), middle-aged

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group (M: 12 months) and old-aged group (O: 24 months). These groups were subdivided into two subgroups of 13 animals each controls and those exposed to SO_2. So our study consisted of six groups: control (YC, MC, OC) and SO_2 groups (YSO_2, MSO_2, OSO_2).

Ten ppm SO_2 was administered to the animals of SO_2 groups in an exposure chamber for one hr (8.00–9.00 a.m)/ day × 7 days/wk × 6 weeks. Control groups were exposed to filtered air in the same chamber for the same period of time. Animals were placed in a 1 m^3 exposure chamber. The gas was delivered to animals to animals via a tube positioned at the upper level of the chamber and distributed homogeneously via a propeller in the chamber. The SO_2 was diluted with fresh air at the intake port of the chamber to yield the desired SO_2 concentration (10 ppm)[12]. Model MRU 95/3-CD apparatus (MRU, MeBgerate für Rauchgase und Unweltschutz GmbH, Heilbronn, Germany) was used to monitor the concentration of SO_2 within the chamber.

At the end of the experimental period, rats were deprived of food for 24 hr and then prepared for experimental procedure under ether anesthesia.

Heparinized blood samples were obtained from ether anesthetized animals by cardiac puncture and plasma was immediately separated after centrifugation (15 min at 4°C, 1,500 × g). Plasma was used for the determination of the vitamin C and Cp. Vitamin C and Cp were determined on the day of the sacrifice of animals. Some of animals were died during the experimental period under ether anesthesia and cardiac puncture. Therefore, number of rats were different between the experimental groups.

Determination of total ascorbate was performed after oxidation of ascorbic acid to dehydroascorbic acid using copper (II) sulfate; the dehydroascorbic acid reacts with acidic 2,4-dinitrophenylhydrazine to form a red bis-hydrazone which was measured at 520 nm[13] by using spectrophotometer (Beckman 26). All vitamin C standards were prepared fresh daily. Plasma ceruloplasmin was measured according to the methods of Schosinky *et al*. by using o-dianisidine dihydrochloride as substrate[14].

Results were expressed as mean with corresponding standard errors. Statistical analysis for significance between the means of the experimental and control groups was checked by Student's t-test. "P" values less than 0.05 were considered to be significant.

The mean vitamin C and Cp values of all groups are given in Table 1. Depending on the age, there was a significant increase in Cp but not in vitamin C. Vitamin C were unaltered with age. Vitamin C was significantly decreased in MSO_2 and OSO_2 groups than in the controls ($p<0.05$). Plasma Cp was significantly decreased in the YSO_2, MSO_2 and OSO_2

Table 1. Vitamin C and ceruloplasmin in the plasma of control and SO_2 exposed rats

Groups	n	Vitamin C (mg/dl) $\bar{X}$ ± SEM	Ceruloplasmin (IU/L) $\bar{X}$ ± SEM
Young control (YC)	12	1.08 ± 0.08	260.33 ± 18.52
Middle-aged control (MC)	10	1.11 ± 0.04 a: n.s	283.09 ± 12.45 a: n.s
Old control (OC)	10	1.14 ± 0.07 b: n.s c: n.s	317.92 ± 10.80 b: p<0.01 c: p<0.01
Young + SO_2 (YSO_2)	10	0.96 ± 0.04 d: n.s	191.84 ± 16.16 d: p<0.01
Middle-aged + SO_2 (MSO_2)	11	0.88 ± 0.07 d: p<0.05 e: n.s	212.27 ± 19.22 d: p<0.05 e: n.s
Old + SO_2 (OSO_2)	12	0.90 ± 0.05 d: p<0.05 f: n.s g: n.s	199.52 ± 20.08 d: p<0.0001 f: n.s g: n.s

a: MC vs YC, b: OC vs YC, c: OC vs MC, d: SO_2 groups vs control groups, e: MSO_2 vs YSO_2, f: OSO_2 vs YSO_2, g: OSO_2 vs MSO_2, n.s: non-significant. The details of the experiments are described in the Materials and Methods section. $\bar{X}$ ± SEM: mean ± standard error of mean. n denotes the number of rats.

groups than in the controls ($p<0.01$) (Table 1).

During evolution living organisms have developed a sophisticated antioxidant system to cope with the deleterious by-products of oxygen reduction. These systems include chemical compounds capable of "scavenging" reactive oxygen species, e.g., vitamins C and E, as well as a series of enzymes specializing in reducing free radicals to more stable species[15]. According to Frei *et al.*[8] vitamin C plays a pivotal role among the chain-breaking antioxidants, and plasma lipid peroxidation is prevented only as long as vitamin C is present. Vitamin C is hydrophilic and functions better in an aqueous environment than does vitamin E. Its role as an antioxidant is indicated by its known free radical scavenging action. As a reducing and antioxidant agent, it directly reacts with various lipid hydroperoxides[10]. Our data showing unchanged vitamin C concentration with age. There are many reports on age-related changes in the vitamin C concentration of rat tissues. Wartanowicz *et al.* pointed out that the concentration of vitamin C decrease in the serum of rats[16]. One past study that reported a significant decrease in vitamin C in the serum of rats at 3, 12 and 24 months of age[17]. These results are inconsistent. This inconsistency might arise from differences in the sex, age, strains and maintenance conditions of rats in the experimental condition used.

Cp is considered preventive plasma antioxidant because it sequester transition metals thereby preventing them from participating in free radical reactions[8]. In the present study, Cp was found to be increased with age. These results are consistent with previous findings[18–21]. However, these findings contradict other author who has reported no age-related changes[22]. Therefore, age-associated changes seem to be different in different experiments. Probably, it depends on the experimental model, i.e., strain of the animals and also on the ages that are studied.

Information on biochemical aspects of SO_2 is very limited. Inhalation of SO_2 (10 ppm daily for 6 weeks) produced a significant decrease in the concentrations of vitamin C in the plasma of SO_2 exposed rats. This finding is in agreement with our previous results. In a previous study[23], we measured vitamin C of plasma of SO_2 (10 ppm daily for 60 days) exposed rats and it was found that exposure to SO_2, decreases vitamin C. The decreased vitamin C might be due to formation of SO_2 radical metabolite. Free radical form of SO_2 is produced in aqueous SO_2 solutions by the action of light, chemical-reducing agents or biochemical-reducing agents[5]. Vitamin C acts as a potent reducing agent. It reduces oxygen-, nitrogen-, and sulphur-centered radicals[24].

In the present study, inhalation of SO_2 10 ppm daily one hour for 6 weeks was found to decrease significantly the Cp in plasma of rats. However, no comparable report is available in the literature on the Cp in rat plasma following exposure to SO_2. The decreased Cp might be due to inactivation of Cp by SO_2. Cp is the major copper containing protein of plasma[9, 25]. It might be possible that the presence of bisulphite (aqueous sulphur dioxide)[26] in the plasma interacts with these copper ions, and leads to inhibition of Cp. Gaseous SO_2 is converted to bisulphite at the surface of the lung[27]. Bisulphite passes into the blood[28].

Our research showed that SO_2 exposure caused a marked decrease in plasma vitamin C and Cp in all experimental groups. The effect of SO_2 on Cp is the highest in the old group. Thus, the observed decrease in vitamin C and Cp in response to SO_2, might constitute protection against superoxide anion elavation. Because the air oxidation of $SO_2^{-}\cdot$ free radical can form superoxide[26]. Vitamin C[24] and Cp react with superoxide[21, 29].

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