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The Effect of Sulfur Dioxide on t-Butyl Hydroperoxide Induced Oxidative Stress in Lung Tissues of Rats

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Abstract: In this study, the effects of sulfur dioxide (SO_2) ,a common pollutant in urban air, on t-butyl hydroperoxide-induced oxidative stress in rat lung tissues were examined. Ten young rats and 10 adult rats were exposed to SO_2 while another 10 young rats and 10 adult rats made up the control groups. Ten ppm SO_2 was administered to the animals of the SO_2 groups in an exposure chamber for one hour a day every day for 6 weeks. The control groups were exposed to filtered air in the same chamber for the same period. At the end of the experimental period, the lung tissues were sonicated and the protein concentration was adjusted to 20 mg/dl. Albumin solution of the same concentration was prepared as the blank. Luminol-dependent chemiluminescence was induced by the addition of t-butyl hydroperoxide in the presence of hemoglobin as the catalyst. The maximum chemiluminescence intensities (MCI) and maximum chemiluminescence time (MCT) detected in both the controls and SO_2 groups were found to be significantly lower and longer respectively than those of the corresponding albumin group. The MCIs detected in the young and adult rats exposed to SO_2 were found to be significantly higher than those of the corresponding control rats. The MCIs detected in the adult rats exposed to SO_2 were found to be significantly higher than those of the young rats exposed to SO_2. The MCTs of the SO_2 groups were significantly decreased in comparison to the respective control groups.

It seems that the exposure of lung tissues to SO_2 increases susceptibility to oxidative stress in rats. The results of this study also suggest that antioxidant protection in lung tissues may diminish with age.

Key Words: Chemiluminescence, SO_2, lung, ageing, rat.

Introduction

Industrial emissions and other urban air pollutants pose a major threat to human health. Sulfur dioxide (SO_2) is a common pollutant of urban air (1). It is highly soluble in an aqueous medium, such as the mucus of the respiratory tract (2). The production of sulfurous acid caused by the contact of SO_2 with moist mucosal surfaces leads to harmful effects (3,4). Concentrations of between 5 and 10 ppm SO_2 cause irritation of the conjuctiva and nasal mucosa (5), while concentrations of 50 ppm and higher lead to severe and massive pathological changes in the respiratory tract and lung parenchyma. SO_2 is known to produce $SO_2^{-\cdot}$ free radical in aqueous environments by the action of light, chemical-reducing agents or biochemical-reducing agents (6).

It has been suggested that hydroperoxide breakdown generates $^1O_2$. This singlet oxygen may be the source of the chemiluminescence (CL) observed during lipid

peroxidation (7). Decomposition of hydroperoxides by metals could yield alcohols, ketones, peroxyl and alkyl radicals which are capable of generating CL. CL arising from transition metal ions or heme compound-catalyzed decomposition of hydroperoxides has been observed. The use of luminol increases the sensitivity of CL (8).

In a previous study, we tested the susceptibility to t-butyl hydroperoxide-induced oxidative stress of lens tissues of rats exposed to SO_2 by using chemiluminescence detection, and it was found that exposure to SO_2 increases susceptibility to oxidative stress (9). In view of our previous findings, our aim was to observe the effects of SO_2 on lung tissue using the same experimental model.

Materials and Methods

Reagents

t-butyl hydroperoxide (t-BuOOH) (70%, w/w aqueous solution), bovine albumin, human hemoglobin and luminol were all obtained from Sigma Chemical Co. (St. Louis, MO, USA). The other chemicals of analytical grade were obtained from Merck Chemical Company (Darmstadt, Germany).

Animals and Procedure

Forty healthy Swiss-Albino male rats (200-400 g) were used in this study. The animals were housed in groups of 4-5 rats in stainless cages in standard conditions (24±2°C and 50±5% humidity) with a 12 hour light-dark cycle. They were separated into 2 equal groups according to age: the young group (3 months) and the adult group (12 months). These groups were divided into 2 subgroups of 10 animals each: the controls and those exposed to SO_2. So our study consisted of four groups: the control (young and adult) and SO_2 groups (young and adult). Ten ppm SO_2 was administered to the animals in the SO_2 groups in an exposure chamber for one hour (8.00-9.00 a.m) a day every day for 6 weeks. The control groups were exposed to filtered air in the same chamber for the same period. The animals were placed in a 1 m^3 exposure chamber. The gas was delivered to the 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 in order to yield the desired SO_2 concentration (10 ppm) (10). A model MRU-35 apparatus was used to monitor the concentration of SO_2 within the chamber.

At the end of the experimental period, the animals were subjected to anaesthesia by inhalation of ether. The whole lung was removed from each animal and washed with cold saline. The lung tissue was sonicated in 50 mM Tris-HCl buffer (pH 7.5) containing 1% ethanol. The tissue homogenate was centrifuged at 20000 x g for one hour. The pellet was then homogenized again in 2 ml of 50 mM Tris-HCl buffer (pH 8.5) containing 150 mM KCl, 10 mM $MgCl_2$ and 1% Triton X-100. Protein was measured by the Lowry method (11), and the protein concentration of the lung tissue homogenate was adjusted to 20 mg/dl by dilution with 50 mM Tris-HCl (pH 8.5). Standard bovine albumin solution at a concentration of 20 mg/dl was prepared in 50 mM Tris-HCl (pH 8.5) buffer using stock bovine albumin solution (100 mg/dl).

Preparation of incubation mixtures

Chemiluminescence (CL) was measured in vials containing 1 ml of incubation mixtures containing 20 mg/dl lung protein homogenate or albumin, 0.47 µM luminol, 45 mg/dl hemoglobin and 150 µM t-BuOOH.

Baseline experiments: We prepared 8 vials for baseline experiments. The content of each vial was: 50 mM Tris-HCL buffer (pH 8.5) with 1% Triton X-100 in the first vial; t-BuOOH in Tris-HCl buffer in the second; t-BuOOH and luminol in Tris-HCl buffer in the third; albumin in Tris-HCl buffer in the fourth; hemoglobin in Tris-HCl buffer in the fifth; homogenate of lung tissue in Tris-HCl buffer in the sixth; and luminol in Tris-HCl buffer in the seventh. The eighth vial was empty.

Assay groups: The incubation mixtures contained 50 mM Tris-HCl buffer (pH 8.5) with 1% Triton X-100, lung homogenate or albumin solution in the presence of hemoglobin, t-BuOOH and luminol at the concentrations mentioned above in a total volume of 1 ml. Five groups of assays were studied in duplicate: 20 mg/dl albumin, 20 mg/dl lung homogenate protein from young and adult control rats together with 20 mg/dl lung homogenate protein from the rats exposed to SO_2.

Chemiluminescence assays

CL measurements were carried out in a Packard 1500 liquid scintillation counter in the single photon mode. Reaction mixtures were placed in a scintillation vial and the CL value was recorded after the reaction initiated by the addition of t-BuOOH. Continuous monitoring of CL was carried out for 3-4 hours at 25°C, measurements being done at 15-min intervals.

Statistics

The data were analyzed by Mann-Whitney U test and two-way analysis of variance in order to identify significant differences. The values are expressed as

mean±SEM. The significance level was taken to be $p<0.05$.

Results

The CL measurements obtained from the baseline experiments did not reflect an increase in any of these groups of assays. Table 1 shows the mean maximum chemiluminescence intensities (MCI) and the mean values of the time at which maximum chemiluminescence intensities occurred (MCT) for all the groups. The MCIs and MCTs in all the groups were compared with those of the control groups. The MCIs detected in both the controls and the SO_2 groups were found to be significantly lower than those of the corresponding albumin (20 mg/dl) group ($p<0.0001$). The MCI values detected in the young and adult rats exposed to SO_2 were found to be significantly higher than those of corresponding control groups ($p<0.05$). The MCI values determined in the adult control animals were found to be significantly higher than those of the young control animals ($p<0.001$). The MCIs determined in the adult rats exposed to SO_2 were found to be significantly higher than those determined in the young rats exposed to SO_2 ($p<0.01$).

The MCTs determined in the controls and young rats exposed to SO_2 were found to be significantly longer than those determined in the albumin group ($p<0.05$). MCT values detected in young rats exposed to SO_2 were not found to be significantly different from those of the young control rats. The MCT values detected in the adult rats exposed to exposed to SO_2 were found to be significantly lower than those of the adult control rats ($p<0.0001$). The differences between the MCTs of the young and adult controls were not found to be significant. The MCTs determined in the adult rats exposed to SO_2 were found to be significantly lower than those determined in the young rats exposed to SO_2 ($p<0.01$).

Discussion

The ability of SO_2 to produce $SO_2^{-\cdot}$ anion radical in an aqueous environment by the action of light, chemical-reducing agents or biochemical-reducing agents reminds us that it takes place in radical reactions in tissues that it reaches. The respiratory tract and lung tissue are the organs that SO_2 can easily incorporate. The harmful effects of SO_2 in these organs have already been reported (6) and oxidative damage may have a role in tissue damage.

Photon counting has been applied to biological systems, and recently evidence has accumulated that CL in the near-infrared region provides useful information on oxidative processes in cells and tissues. The advantages of the technique are that it is non-invasive and allows continuous monitoring (12). Luminol-amplified CL is a sensitive and reproducible technique for the continuous monitoring of free radicals generated during the treatment of tissues with organic hydroperoxide. Organic hydroperoxides induce CL in experimental models in the presence of hemoglobin (12-14). In our experimental system, we induced CL by t-butyl hydroperoxide in lung homogenates in the presence of hemoglobin and luminol. Hemoglobin catalyzes the cleavage of organic hydroperoxides, leading to the generation of free radicals which initiate lipid peroxidation (13,15). Free radical production correlates with increased CL (13).

CL production is also dependent on protein concentration (9, 14-16). Therefore, we used albumin as the blank and the protein concentration of each sample was adjusted to the same level (20 mg/dl) to prevent possible interfering effects of proteins on

Table 1. Maximum chemiluminescence intensities (MCI) and maximum chemiluminescence time (MCT) detected in albumin, control and SO_2 exposed groups.

Groups	n	MCI (cpm) X ±SEM	MCT (min) X ±SEM
Albumin (20 mg/dl)	10	453.66 ± 8.95	16.00 ± 1.25
Young control	10	275.63 ± 9.60	27.00 ± 2.00
Young rats exposed to SO_2	10	305.72 ± 9.05	22.50 ± 2.50
Adult control	10	324.93 ± 9.17	30.00 ± 0.00
Adult rats exposed to SO_2	10	388.12 ± 8.25	15.00 ± 0.00

The details of the experiments are described in the Materials and Methods section.
X ± SEM: mean ± standard error of mean.
n denotes the number of rats.

chemiluminescence formation. The higher CL intensities detected in the albumin solutions than in the lung tissue homogenates of the controls and the rats exposed to SO_2 were probably caused by a lack of antioxidant systems in the albumin solutions. Tissue homogenates are known to include antioxidant enzymes (glutathione peroxidase, catalase and superoxide dismutase) and other antioxidants (vitamin C, vitamin E, urate, taurine and glutathione). t-Butyl hydroperoxide induces oxidative stress and these tissue antioxidants, which were not present in the pure albumin solution, reduce oxidant attack more effectively-these were observed as low MCI and high MCT values in the tissue homogenates -than pure albumin solution.

In our previous studies, we showed that the antioxidant properties of a substance are defined by MCIs being lower and MCTs being longer (15). If the antioxidant property is strong, CL formation is prevented to some degree. This is observed by elongation of the period in which MCI occurs (MCT). The MCTs of the rats exposed to SO_2 were shorter and this was accompanied by increased MCI. These findings support the idea that the antioxidant mechanisms of the rats exposed to SO_2 decreased.

CL intensities showed a marked increase in all the experimental groups following SO_2 exposure compared to the controls. Susceptibility to oxidative stress was defined by CL intensities. Those detected in the lung tissue homogenates of adult rats exposed to SO_2 were found to be higher than those in the lung tissue homogenates of young rast exposed to SO_2. This finding is in agreement with the report of Delacourt et al (17). In our previous study, exposure to SO_2 led to increased susceptibility to oxidative stress in lens tissues of rats, which can be explained by the formation of free radicals originating from SO_2 (9). The findings of this study for young and adult rats exposed to SO_2 support the previous lens study. Hypoxia, caused by SO_2 may be responsible for this phenomenon. Archer (18) reported that lung tissue radical levels measured by luminol-enhanced CL decreased in relation to the degree of alveolar hypoxia.

Our results also show an age-dependent increase in CL intensity, which is an indicator of oxidative stress in lung tissue homogenates in both the control and SO_2 groups. In our study, the increased susceptibility to oxidative stress observed in adult rats also explains the decreasing of antioxidant defence mechanisms by ageing. Our data are consistent with a previous study (19).

SO_2 is an air pollutant that has significant harmful effects on health. In this study, SO_2 seemed to increase the susceptibility of the lung tissues of rats to oxidative stress. In other words, SO_2 decreased the antioxidant defense of the lung tissue, which probably caused pathological changes.

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