The effects of thinner inhalation on superoxide dismutase activities, malondialdehyde and glutathione levels in rat lungs

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Abstract

Background: Recent years’ usage of thinner by the young generation as a drug constitutes a serious problem in the society. Due to common usage in the industrial sector, most people are affected from the manufacturing process to the consuming phase.

Aim: Because of these reasons, this project has been preferred to research the effects of thinner on oxidant and antioxidant status.

Methods: Totally 46 rats were included in the study. Thirty six rats were separated into six groups with 10 rats in a control group. The first group inhaled thinner for 2 weeks, and the other groups were exposed to thinner for 4, 6, 8, 10 and 12 weeks for 1 h twice a day. On the mentioned duration, rats were autopsied. Lung tissues malondialdehyde (MDA), glutathione (GSH) levels and superoxide dismutase (SOD) activities were determined to designate the oxidant–antioxidant balance.

Results: We observed an increase in MDA values both in the acute and the subacute periods. In the chronic period by the consuming of lipid peroxidation products, MDA values decreased and as the oxidative stress continued MDA values again increased. We observed that especially GSH values that has antioxidant feature, decreased until 6 weeks in order to compensate lipid peroxidation products. In the consuming period of lipid peroxidation, the values became fixed and later, these values again increased. There was no relationship between the changing values of MDA and SOD.

Conclusions: Thinner is an agent that causes oxidative stress and inhalation of high doses of thinner causes harm to the respiratory system. As there are few reports in the literature on long-term effects of thinner inhalation, more studies might be necessary.

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Keywords: Thinner inhalation; Lipid peroxidation; Antioxidants

1. Introduction

Cellulose thinner is composed of more than one organic solvent. In general, it is present in the content of industrial dyes, polishes, glues and dry cleaning liquids [1,2]. Because of this widespread utilization, many people are exposed to the solvents in thinner during production or consumption via skin or respiration. The inhalation of solvents in thinner, in an amount that does not cause loss of consciousness, results in euphoria and relaxation similar to alcohol ingestion. On the other hand, inhalation exceeding the maximal dose can cause central nervous system depression, unconsciousness, respiratory arrest and even death [3].

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There is some evidence that organic solvents may express their toxicity by the way reactive oxygen species (ROS) induced cell damage [4,5]. The reactive species, such as superoxide anion and hydroxyl ion, are believed to initiate this process [6].

The aim of this experimental study was to investigate the harmful effects of thinner on the rat lung: peroxidative changes during 12 weeks of inhalation. With this intent, we measured lipid peroxidation, often referred as thiobarbituric acid reactive substance (TBARS) which is composed of malondialdehyde (MDA) in rat lungs. Also, antioxidant enzyme superoxide dismutase (SOD) and major cellular defence mechanism against free radical-induced damage, reduced glutathione (GSH), were examined in order to obtain the changes in antioxidant defence during the inhalation period in the lungs.

2. Materials and methods

2.1. Animals and inhalation

Male Wistar Albino rats weighing 200–250 g were used throughout the experiment. Six test groups, each consisting of six rats, were used. They were maintained under controlled conditions: constant temperature (20–22 °C), volume (189,000 cm³) and pressure at sea level (760 mm Hg). The rat groups were placed in a specially designed cage (size 90 × 60 × 35 cm, air ventilation) and had inhaled a controlled amounts of thinner. The first group inhaled thinner for 2 weeks, and the other groups were exposed to thinner for 4, 6, 8, 10 and 12 weeks for 1 h twice a day. The common thinner in our industry which is composed of toluene (63%), acetone (13%), isobutyl acetate (10%), isobutanol (7.5%) and butyl glycol (65%) was used in our

![Graphs showing effects of thinner inhalation on malondialdehyde, glutathione levels and superoxide dismutase activities in rat lungs.](image)

Fig. 1. The effects of thinner inhalation on malondialdehyde, glutathione levels and superoxide dismutase activities in rat lungs (wk: week). *Significantly different from control group, p < 0.05.
experiments. The concentrations of the solvents in the thinner vaporized into the cage gas detector were as follows: toluene 2800–3000 ppm, acetone 500–600 ppm, isobutyl acetate 7000–8000 ppm, and isobutanol 6000–7000 ppm. Thinner was vaporized into the cage with a millipore pump at a constant pressure twice per day for 1 h for 5 days. The control group was only exposed to fresh air ($n=10$).

At the end of the inhalation period, rats were anesthetized and decapitated. Lungs were removed and cut into two pieces. One lung was washed in 0.9% NaCl and kept in ice. Tissues were homogenized with cold 1.15% KCl to make a 10% homogenate (w/v). Tissue lipid peroxide levels, expressed in terms of MDA, were determined according to the method of Buege and Aust [7]. The results were expressed as nmol/100 mg protein. Tissue GSH was measured 5,5'-dithiobis-(2-nitrobenzoate) at 412 nm according Ellman [8], and the results were expressed as nmol/mg protein. The protein concentrations of tissue homogenates were determined by the method of Lowry et al. [9]. Cu–ZnSOD activity was measured kinetically by the method of Sun et al. [10]. The activity was expressed as U/mg protein.

Statistical analysis was performed by the one-way analysis of variance (ANOVA) method. Student’s t-test was used for group comparisons with significance at the $p<0.05$.

3. Results

Fig. 1 shows the effects of thinner inhalation on MDA levels, SOD activities and GSH levels in rat lungs. MDA levels of the rats, which inhaled thinner for 8 and 12 weeks, were found to be significantly higher, when compared to the control group ($p<0.05$). MDA levels of rats were significantly increased when compared to the inhalation period ($p<0.0001$). The highest levels of MDA were found in the 12th week rats. No statistical difference was found in tissue GSH levels and SOD activities between the weeks ($p>0.05$).

4. Discussion

Inhalation of the solvents commonly present in the flammable chemicals may result in acute effects in the nervous system and behaviors of the living structures. Acute effects of the flammable organic solvents were compared with that of classical central nervous system depressing drugs, such as barbiturates, benzodiazepins and ethanol, and it was shown that they act similarly in their biphasic effects in the motor activity, deterioration in the psychomotor performance, and anticonvulsant efficacy [11]. On the other hand, chronic exposure to these solvents was noted to result in loss of IQ, changes in the cerebellar morphology, atrophy, loss of weight in the prostate and testis, hyperkinesia, problems in fertility, congenital defects, alternations of the pulmonary function, renal dysfunction and finally death [3].

In the study by Saygý et al., rats were exposed to thinner inhalation for 5 weeks and it was seen that rats had somnolence, slowness of motion, difficulty in gait, increased frequency of defecation due to increased intestinal motility, and stable prolapsus. Some rats had harsh respiration at the fourth week of the inhalation this had been observed to revert to normal following the cessation of inhalation [12].

Various studies showed that the cellular damage caused by thinner were in fact due to free oxygen radicals (FORs) [13]. FORs result in lipid peroxidation which in turn culminates in the damage of the biological membranes. Unfortunately, our literature survey was not successful in finding any study which examined in the long term, the effects of thinner on pulmonary tissue. The studies were about brain, liver and reproductive organ tissues.

The study from Halifeoğlu et al. examined the workers who had been exposed to thinner for 10 years while working in the dye industry, and the levels of MDA, Glutathione peroxidase (Gpx) and SOD were determined from the blood samples; MDA levels were found to be significantly higher than in the control group [13]. Considering the increase in the levels of SOD and Gpx together with MDA, this could be secondary to a compensatory mechanism developed as a result of workers’ longstanding exposure to thinner. In their study, Rahman et al. [14] showed that while GSH level measured in the fluid lining, the pulmonary epithelium increased in chronic smokers, this level decreased in acute smokers. Ulakoğlu et al. [15] measured the levels of MDA, SOD and GSH in rats that had inhaled a high concentration thinner for 5 weeks in their study and found that the levels of SOD
and GSH decreased but the level of MDA increased significantly during the period of inhalation. This decrease could be the result of antioxidant defense mechanism.

In our study, we observed a tendency for increase in the levels of MDA during the period of thinner inhalation. However, MDA levels decreased significantly at the 10th week and increased again significantly after the 12th week. The 10th week was a time when lipid peroxidation products were depleted and oxidant–antioxidant balance was achieved. GSH was produced by the body between the 6th and 10th weeks for the compensation of initial decrease in order to maintain the balance again. Furthermore, GSH levels began to increase again after the 12th week in order to balance the products of lipid peroxidation which have been released as a result of continuing oxidative stress. SOD levels could not increase or decrease significantly to balance oxidant–antioxidant mechanisms (paths). Our interpretation for this result is that GSH provided a more effective antioxidant mechanism than SOD against oxidant mechanisms. These findings confirmed that thinner inhalation resulted in cell damage by inducing oxidative stress in the lung tissue.

We have also observed that long-standing thinner inhalation resulted in emphysema similar to chronic obstructive pulmonary disease in smoking. These findings suggest that pulmonary destruction can be earlier and more severe if workers are exposed to both thinner inhalation and smoking damage at the same time rather than a situation where only one is present.

For this reason, thinner abuse is a serious public health problem which requires preventive precautions and intense education. Drugs, which increase the mucociliary activity along with their antioxidant effects, have been routinely used in chronic smokers. We hope that our study will shed light on further studies in which the preventive effectiveness of such drugs is examined on persons who are exposed to thinner because of their occupation.

References