Antioxidant enzymes and lipid peroxidation in adolescents with inhalant abuse

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Glue and thinner sniffing is a serious medical problem since the volatile constituents of these substances can lead to severe damage to bone marrow, liver, kidney, brain, and heart. The exact mechanisms responsible for tissue and organ damage in children with inhalant abuse have not yet been determined. In this study, we aimed to determine the levels of antioxidant enzymes and malondialdehyde in children with inhalant abuse. Erythrocyte and plasma glutathione peroxidase (GSH-Px) activities, erythrocyte superoxide dismutase (SOD) activity, and erythrocyte and plasma malondialdehyde (MDA) levels were measured as biological markers of oxidative damage and lipid peroxidation in 37 glue and thinner (inhalant) abusers. The levels were compared with those of the 27 well-matched healthy adolescents. Erythrocyte SOD activity and erythrocyte and plasma MDA levels were significantly higher, whereas erythrocyte and plasma GSH-Px activities were significantly lower in children with inhalant abuse when compared to controls ($p<0.01$). These findings imply that chronic inhalation of volatile substances can alter the levels of antioxidant enzymes including SOD and GSH-Px, and can increase lipid peroxidation in adolescent abusers.

Key words: antioxidant system, glue, glutathione peroxidase, inhalant abuse, lipid peroxidation, malondialdehyde, oxidative damage, superoxide dismutase, thinner, volatile substance.

Inhalant abuse is defined as the intentional inhalation of a volatile substance in order to achieve a euphoric state. It is primarily practiced by children and teenagers, with onset occurring predominantly at 6 to 8 years of age, and with a peak prevalence at the age of 14 to 15 years in the United States[1]. Glue and thinner sniffing are the most common forms of inhalant use due to their low cost and ready accessibility.

The best characterized components of volatile glues and thinner are aromatic hydrocarbons such as toluene, n-hexane, benzene, ethyl acetate, and butyl acetate, and minor proportions of other solvents[2]. Glue and thinner sniffing is, thus, a serious medical problem since these components can cause severe damage to bone marrow, liver, kidney, brain and heart.

The exact mechanism(s) responsible for tissue and organ damage in children with inhalant abuse have not yet been determined. A few animal and human studies have shown that toluene and benzene can cause oxidative damage by changing the activities of antioxidant enzymes[3-5]. The effects of thinner on lipid peroxidation and antioxidant enzymes in people working with paint thinner have been investigated in a study[5]. However, to our knowledge, the status of antioxidant enzyme systems and lipid peroxidation in children with inhalant abuse has not been studied before. In this study, therefore, we aimed to determine the levels of antioxidant enzymes (superoxide dismutase and glutathione peroxidase) and malondialdehyde in children with inhalant abuse, considering the possible role of oxidative damage and lipid peroxidation in the pathophysiology of tissue damage in abuse of volatile substances.

Material and Methods

This study was performed at the Ankara Education and Rehabilitation Center for Children, where impatient and outpatient

substance abuse treatment and prevention programs are conducted, between September 2000 and May 2001.

The study group consisted of 37 boys, aged between 11 to 19 years (mean 15.3±2.4 years), who were admitted by their parents or relatives because of chronic abuse of glue and thinner sniffing. The presence and duration of a chronic inhalation of a volatile substance were confirmed after interview with each case, and the diagnosis of addiction of a volatile substance inhalation was established according to the DSM-IV criteria[6]. These subjects had halted their abuse at least one month previously (range 1 to 3 months, mean 2.3±0.7 months), and the duration of abuse before rehabilitation was between 2 to 5 years (mean 3.2±0.6 years). All subjects were tobacco smokers, and none had taken any antioxidant medications (vitamin C, vitamin E, selenium, etc.) prior to or during the study. The parents and official authorities were informed about the study and an informed consent was obtained from the subjects and/or their parents.

Twenty-seven healthy volunteer adolescents of similar age (mean 15.8±2.4 years) without any addiction were chosen as the control group. A brief history was taken, a complete physical examination was performed, and blood samples for complete blood count and renal and liver function tests were obtained for each individual in the study and control groups. Subjects with any signs or symptoms of any acute or chronic illness or with abnormal biochemical test were excluded from the study.

The effects of volatile substances on the antioxidant system were evaluated by measuring erythrocyte superoxide dismutase (SOD) and erythrocyte and plasma glutathione peroxidase (GSH-Px) activities. Membrane lipid peroxidation was assessed by measuring erythrocyte and plasma malondialdehyde (MDA) levels. Blood samples were withdrawn by venipuncture to the glass tubes containing tripotassium ethylenediaminetetraacetic acid (EDTA), and were centrifuged immediately for six minutes with 1340 G at 4°C to separate plasma. The buffy coat was removed, and the remaining erythroctes were drawn from the bottom and washed three times in cold isosmotic phosphate-buffered saline (9.0 g/L NaCI). Spectrophotometric analysis of SOD and GSH-Px in erythrocytes and of GSH-Px in plasma was performed as described previously[7,8]. Plasma and erythrocyte MDA levels were measured using a thiobarbituric acid reactive substance (TBARS) method[9]. After the reaction of TBARS, the reaction product was measured spectrophotometrically at 532 mm.

Statistical comparison of the data obtained from the study and control groups was made using Mann-Whitney U test.

Results

Erythrocyte and plasma activities of antioxidant enzymes and MDA levels are shown in Table I. Erythrocyte SOD activities were significantly higher in the study group when compared to the control group (582.3±184.0 U/gHb versus 248.7±58.5 U/gHb, $p<0.001$). Erythrocyte and plasma GSH-Px activities were significantly lower in the study group (22.6±6.1 versus 40.2±5.2 U/gHb, $p<0.001$; and 2.30±0.32 versus 4.8±0.9 U/ml, $p<0.001$, respectively). Erythrocyte and plasma MDA levels were significantly higher in the study group (6.3±1.1 versus 3.2±0.4 nmol/ml, $p<0.001$; and 0.6±0.5 versus 0.2±0.04 nmol/ml, $p<0.001$, respectively).

Discussion

Free radicals are continuously produced during aerobic metabolism[10]. Oxidative damage caused by free radicals is counteracted by a number of enzymes and vitamins[11]. The imbalance between

Table I. Erythrocyte and Plasma Activities of Antioxidant Enzymes and MDA in Teenagers with Inhalant Abuse (Study Group) and Healthy Control Group

Parameters	Study group (n=37)*	Control group (n=27)*	p value
Erythrocyte SOD (U/gHb)	582.3±184.0	248.7±58.5	<0.001
Erythrocyte GSH-Px (U/gHb)	22.6±6.1	40.2±5.2	<0.001
Plasma GSH-Px (U/ml)	2.3±0.3	4.8±0.9	<0.001
Erythrocyte MDA (nmol/ml)	6.6±1.1	3.2±0.4	<0.001
Plasma MDA (nmol/ml)	0.6±0.5	0.2±0.04	<0.001

SOD : superoxide dismutase. GSH-Px : glutathione peroxidase. MDA : malondialdehyde.
* Values are given as mean ± SD.

the rate of free radical production and the effect of protective antioxidatiants leads to oxidative damage, which is also known as oxidative stress.

Superoxide dismutase (SOD) is an enzyme extensively used as a biochemical indicator of pathological states associated with oxidative stress. At the first step of the defense system against oxidative stress, it catalyzes dismutation of the superoxide anion (O_{2-}) into hydrogen peroxide (H_2O_2). H_2O_2 is one of the most active oxygen species[12]. A significant increase in SOD activity in erythrocytes in our study may have occurred in order to neutralize the excess superoxide anions originating from thinner and glues. Indeed, benzene metabolites are known to produce oxidized species and reactive oxygen. Furthermore, inhibition of benzene metabolism by toluene increases the reactive oxygen13. The more reactive oxygen is produced, the more the activity of SOD is increased in order to convert it into H_2O_2.

At the second step of the antioxidant defense system, GSH-Px and catalase independently degrade H_2O_2 to water. Any increase in SOD activity should, therefore, produce an excess H_2O_2 that must be efficiently neutralized by either GSH-Px or catalase[14]. Halifeoğlu et al.[5] accordingly showed increased SOD and GSH-Px levels in people working with paint thinner. Both erythrocyte and plasma GSH-Px levels were, however, significantly lower in adolescents with inhalant abuse in our study. This finding indicates that excess of H_2O_2 cannot be neutralized efficiently in these subjects, probably due to the exhaustion of the enzyme by H_2O_2 and lipid peroxides. If the amonunt of free radicals that cannot be efficiently neutralized after an oxidative stress reaches a critical level, then lipid peroxidation occurs subsequently. MDA is one of the most commonly used indicators of lipid peroxidation, and it was also significantly increased in our study group, indicating an increased risk of cell membrane damage.

Results of the present study imply that chronic inhalation of volatile substances can alter the levels of antioxidant enzymes including SOD and GSH-Px, and can increase lipid peroxidation in adolescent abusers. The role of antioxidant therapy or supplementation in children with inhalant abuse remains to be determined considering the possible consequences o oxidative damage and lipid peroxidation, including the risk of severe damage to various tissues and organs such as bone marrow, liver, kidney, brain, and heart.

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