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Short Communication

HOW VOLATILE ORGANIC COMPOUNDS AFFECT FREE RADICAL AND ANTIOXIDANT ENZYME ACTIVITY IN TEXTILE WORKERS

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Various effects of exposure to volatile organic compounds (VOC) have recently become an important issue because of their widespread use in industry. The aim of this study was to determine the effect of volatile organic solvents commonly used in textile paint industry on free radical levels and the antioxidant enzyme system in textile workers. The study included twenty exposed workers and twenty controls. Blood samples were taken after an overnight fast of 10 to 12 hours. Malondialdehyde (MDA) and total antioxidant capacity (TAC) were studied in serum while super oxide dismutase (SOD) activity was studied in erythrocytes. Statistical analysis was performed using the ANOVA and Kruskall-Wallis analysis tests. MDA and SOD were significantly higher in textile workers than in controls ($p<0.01$). In contrast, no significant difference was observed between them for TAC ($p>0.05$).
Elevated MDA levels in textile workers may indicate increased lipid peroxidation as a result of long-term exposure to organic solvents, whereas elevated SOD activity suggests that the antioxidant system was activated to counter lipid peroxidation. The results suggest that textile workers occupationally exposed to volatile organic solvents make a risk group and require more frequent periodic examinations.

KEY WORDS: *occupational exposure, malondialdehyde, total antioxidant capacity, super oxide dismutase, biomonitoring*

Free radicals are continuously produced during aerobic metabolism. Oxidative damage caused by free radicals is counteracted by a number of enzymes and vitamins. An imbalance between the rate of free radical production and the effect of protective antioxidants leads to oxidative damage, which is also known as oxidative stress (1). The toxic effects of volatile organic compounds (VOCs) are caused by reactive oxygen species (ROS). Free radicals are highly reactive species that are involved in cellular damage. Reactive intermediates are also generated by the cytochrome P450 enzymatic system. These intermediates may bind covalently to proteins, lipids, DNA or RNA, and may inactivate receptors and proteins, damage cellular membranes or initiate mutagenic reactions (2). ROS are believed to cause lipid peroxidation, which in turn damages biological membranes (3). Antioxidants such as enzymatic and nonenzymatic defence systems are necessary to prevent cellular damage (4).

Previous studies on textile workers investigated the association between exposure and health effects, yet little is known about biomarkers of these disorders. The levels of MDA, SOD, and TAC in textile workers have not been extensively studied. Therefore, the aim of this study was to examine the effects of volatile organic compounds on lipid peroxidation and antioxidant enzyme activity in peripheral blood of exposed textile workers.

MATERIALS AND METHODS

Subjects

The exposed group included twenty male textile workers, and twenty healthy, male, age-matching subjects were taken as controls (Table 1). All the workers were asked to answer a questionnaire that included questions about age, smoking habit, health, working years as a textile worker, and the use of protective gloves and personal respiratory equipment at work. There were 9 smokers 11 non-smokers at both group. The study was explained to workers and take permission from local ethics committee. Blood samples were taken from each subject after 10 to12 hours of fasting using vacutainers. After collection, the samples were coded and transported to the laboratory for testing. Sera were separated and used without any treatment. Erythrocytes were washed with a 0.9 % NaCl solution and centrifuged for 10 min. Washing was repeated three times. After complete lysis, cellular particles were removed by centrifugation and the haemolysate was used to determine SOD activity.

Measurement of MDA levels

Serum lipid peroxide levels were determined by measuring malondialdehyde, which is an end-product of lipid peroxidation. We used the spectrophotometric method described by Jain (7). The color intensity of the complex obtained by the reaction of MDA and thiobarbituric acid is proportional to the concentration of MDA, and was determined at 532 nm. The concentration of MDA is expressed as nanomole of MDA per millilitre of serum.

Measurement of erythrocyte SOD activity

The activity of superoxide dismutase was measured using the RANSOD kit (Randox Laboratories Ltd., UK). The role of superoxide dismutase is to accelerate the dismutation of the toxic superoxide radical O^{-}_{2} to hydrogen peroxide and molecular oxygen. This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye.

Superoxide dismutase activity is then measured by the degree of inhibition at 505 nm. Haemoglobin was measured using an automatic whole blood counter. The results were expressed as SOD activity per gram of haemoglobin.

Measurement of total antioxidant capacity

Total antioxidant capacity was measured by the reaction of antioxidants in the sample with a defined amount of added hydrogen peroxide (H_2O_2) following the standardised procedure (enzyme-labelled test). Antioxidants in the sample eliminate a certain amount of added hydrogen peroxide. The residual H_2O_2 is determined colourimetrically through enzyme reaction which involves the conversion of TMB to a coloured product. After addition of a stop solution, the samples were measured at 450 nm in a microtitre plate reader. The difference between the applied and measured peroxide concentration in a defined time period is proportional to the reactivity of the antioxidants in the sample (antioxidant capacity).

Statistical analysis

Means and standard deviation were calculated for each parameter and the statistical analysis performed using SPPSS version 10.00 and Microsoft Excel. We used the Kruskall-Wallis variance test and set the value of $p<0.05$ as statistically significant.

RESULTS AND DISCUSSION

None of the textile workers in this study used gloves and/or protective respiratory equipment. All workers had at least five years of working experience in this occupation. Nine were smokers and 11 non-smokers in both the exposed and control group (Table 1). The MDA level was significantly higher in textile workers than in controls ($p<0.01$) and so was the SOD activity ($p<0.01$). However, no statistical difference was observed between textile and control group in TAC ($p>0.05$) (Table 2). Our data indicate that smoking is not a confounder for the association between solvent exposure and changes in blood parameters.

Elevated MDA levels in the textile group may indicate an increased lipid peroxidation as a consequence of long-term occupational exposure to organic solvents. Higher SOD activity in the textile group compared to controls suggests that the antioxidant system is activated to counter lipid peroxidation.

Organic solvents are a chemical class of compounds that are used routinely in industries. They share a common structure (at least one carbon and one hydrogen), low molecular weight, lipophilicity, and volatility, and they exist in liquid form at room temperature (5) at which they readily evaporate. After

Table 1 *Characteristics of the groups studied*

Parameter	Textile workers (n=20)	Controls (n=20)	p
Age / year	31.75±2.12	31.35±3.12	>0.05
Height / m	1.697±6.06	1.77±6.92	>0.05
Weight / kg	69.5±9.95	80.05±11.93	>0.05
Smoking / year	10±5.97	9.88±6.25	>0.05
Cigarettes per day / number	18.57±3.77	21.87±7.52	>0.05
Working years / number	8.27±3.87		
Smokers/Non-smokers	9/11	9/11	

Data are presented as mean ± SD

Table 2 *MDA, SOD, and TAC in textile workers and controls*

Parameter	Textile Group (n=20)	Control Group (n=20)	p
MDA[a] /nmol mL^{-1}	4.35±0.46	1.52±0.45	<0.01
SOD[b] / U g^{-1} Hb	1234.18±26.39	1045.19±14.75	<0.01
TAC[a] / μmol L^{-1}	280.18±50.84	244.72±57.11	>0.05

Data are presented as mean ± SD
[a] *In serum*
[b] *In erythrocyte*

being inhaled, they rapidly pass from the alveoli into the blood. The liver is the site of most solvent metabolism, specifically via the cytochrome P450 mixed function oxidase system, which varies in individuals by ethnicity and age. Cytochrome P450 was shown to yield very high levels of reactive oxygen species (7). Few studies have evaluated the production of reactive oxygen intermediates by human microsomes or the influence of cytochrome P450 in particular. Experiments were carried out to evaluate the ability of CYP1A1, 1A2, 2B6, and 3A4 to consume NADPH, reduce iron, and catalyse production of reactive oxygen species. CYP3A4 is present in high amounts in human liver microsomes and is catalyses the formation of reactive oxygen species; this contribution in the overall ability of human liver microsomes to generate active oxygen species may be very important (8).

ROS-induced lipid peroxidation is an oxidative process associated with membrane lipid destruction. MDA is formed as its end product. SOD is an enzyme extensively used as indicator of oxidative stress. It is the first step of the defence system against oxidative stress, and catalyses the dismutation of superoxide anions (O_2^-) into hydrogen peroxide (H_2O_2). H_2O_2 is one of the most active oxygen species (9).

A significant increase in SOD activity in erythrocytes in our study may have occurred in order to neutralise the excess superoxide anions caused by volatile organic compounds such as benzene, toluene, and thinners. In addition, benzene metabolites are known to produce oxidised species and reactive oxygen (10). Furthermore, inhibition of benzene metabolism by toluene increases the production of reactive oxygen, which in turn increases the activity of SOD in order to convert it into H_2O_2. To our knowledge no similar study has examined the effect of antioxidant enzymes and free radicals in exposed textile workers. Halifeo lu et al. found increased MDA and SOD activity in workers occupationally exposed to paint thinners (11). Increased SOD activity was reported in adolescents with inhalant abuse (12).

The antioxidant system has many components. Antioxidant capacity may give more relevant biological information than measurements of individual

components, as it includes the cumulative effect of all antioxidants present in plasma and body fluids (13). As the results of this study did not reveal statistically significant differences in TAC between the textile workers and controls, it is possible that many mechanisms synergistically counteract oxidative damage, but it remains to be investigated in a much larger population. Based on the results obtained in this study we can conclude that textile workers occupationally exposed to volatile organic solvents represent a risk group, and require more frequent periodic examinations. It would also be reasonable for these workers to take antioxidant supplements with their regular diet and to use personal protective equipment at work (at least protective gloves and respiratory protection).

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Sažetak

KAKO HLAPLJIVI ORGANSKI SPOJEVI UTJEČU NA SLOBODNE RADIKALE I AKTIVNOST ANTIOKSIDACIJSKIH ENZIMA U TEKSTILNIH RADNIKA

Štetni učinci izloženosti hlapljivim organskim spojevima u novije su vrijeme razlog zabrinutosti zbog njihove sve veće uporabe u industriji. U ovome su istraživanju procjenjivane razine slobodnih radikala te aktivnost sustava antioksidacijskih enzima u uzorcima periferne krvi radnika zaposlenih u pogonima za bojenje tkanina izloženih hlapljivim organskim otapalima. Istraživanje je provedeno na dvadeset profesionalno izloženih ispitanika te dvadeset ispitanika kontrolne skupine. Uzorci periferne krvi svim su ispitanicima uzimani u jutarnjim satima, nakon 10 do12 sati gladovanja. Koncentracije malondialdehida (MDA) i ukupni antioksidacijski kapacitet mjereni su u serumu, a aktivnost super oksid dismutaze (SOD) u eritrocitima. Statistička analiza provedena je primjenom ANOVA i Kruskall-Wallisova testa. U skupini tekstilnih radnika utvrđena je značajno povišena razina MDA u usporedbi s kontrolom ($p<0,01$). Aktivnost SOD u tekstilnih radnika također je bila viša nego u kontroli ($p<0,01$). Nasuprot tomu, između dviju skupina nije uočena statistički značajna razlika u ukupnom antioksidacijskom kapacitetu. Povišene koncentracije MDA u tekstilnih radnika mogu upućivati na povećanu lipidnu peroksidaciju koja nastaje kao posljedica višegodišnje profesionalne izloženosti organskim otapalima, dok povećana aktivnost SOD upućuje na aktiviranje antioksidativnih enzima čija aktivnost raste zbog uklanjanja produkata nastalih u procesu lipidne peroksidacije. Na osnovi dobivenih rezultata zaključujemo da tekstilni radnici profesionalno izloženi hlapljivim organskim otapalima predstavljaju skupinu s povišenim zdravstvenim rizikom te stoga moraju biti pod medicinskim nadzorom koji uključuje češće periodičke preglede.

KLJUČNE RIJEČI: *profesionalna izloženost, malondialdehid, ukupni antioksidacijski kapacitet, super oksid dismutaza, biomonitoring*

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