Indian Journal of Biochemistry & Biophysics
Vol. 41, February 2004, pp. 40-44

Combined effect of alcohol and cigarette smoke on lipid peroxidation and antioxidant status in rats

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Received 21 August 2003; revised 16 January 2004

The effect of long-term administration of alcohol and cigarette smoke independently and both in combination on lipid peroxidation and antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione-S-transferase (GST) was studied in liver, kidney, heart and lungs of albino rats. The levels of peroxidation products viz., malondialdehyde, hydroperoxides and conjugated dienes were increased in all the tissues of alcohol administered and smoke-exposed rats. Activities of SOD and CAT were decreased in alcohol-treated and alcohol and smoke combination groups, but increased in smoke-exposed group. Activities of GPx and GST have shown an increase, while concentration of reduced glutathione was found decreased in all the three groups.

Keywords: Alcohol, cigarette smoke, lipid peroxidation, antioxidant status

Alcohol consumption in humans is a serious health hazard, and the liver is major organ susceptible to it[1]. Administration of ethanol results in a variety of changes in the liver and acute ethanol ingestion causes fatty liver and hypertriglyceridemia[2]. It may also alter normal function and composition of lipid membranes[3]. Hepatic cirrhosis is a major cause of death in chronic alcoholics[4].

Cigarette smoke produced by incomplete combustion of tobacco generates a high free radical load *in vivo*[5]. It contains various chemical compounds including biphenyl and polycyclic aromatic hydrocarbons which could initiate and promote oxidative damage[6,7]. The oxidants[8] present in cigarette smoke are involved in the pathogenesis of diseases of lungs and vascular system[9]. Serum levels of high-density lipoproteins (HDL) are generally lower in smokers, compared to those of non-smokers[10]. Prolonged exposure to cigarette smoke, besides decreasing HDL-cholesterol, increases low-density lipoproteins (LDL) and very-low-density lipoproteins (VLDL) levels[11]. LDL can be altered by diet and certain reactive chemicals like malondialdehyde (MDA)[12]. Plasma MDA level is high in smokers since cigarette smoke contains reactive oxygen radicals[13] and acetaldehyde[14], which can increase lipid peroxidation. Cigarette smoking is an exogenous factor which is reported to cause a 3-fold increase in the incidence of myocardial infarction (MI)[15].

Reactive oxygen species (ROS) formed in the body as a result of normal metabolic reactions, exposure to ionizing radiation, cigarette smoke, environmental pollution, and by the influence of several xenobiotics etc. are implicated in several diseases, including cancer[16]. ROS damage DNA, proteins, carbohydrates and lipids and affect enzyme activity and the genetic machinery. However, biological systems possess a number of mechanisms to remove free radicals. The integrated antioxidant system, which scavenges free radicals, has an important role in the removal of free radicals[17]. In the present study, we have examined the combined effect of alcohol and cigarette smoke on lipid peroxidation and antioxidant status in albino rats.

Materials and Methods

Male albino rats of Sprague Dawley strain weighing 200-250 g were maintained under environmentally controlled conditions with free access to standard food (Lipton, India) and water. Rats were divided into four groups: Group I: normal diet (control group); Group II: normal diet +18% alcohol (4 g alcohol/kg body wt/day) orally (alcohol-treated group); Group III: normal diet+cigarette smoke (smoke-exposed group); and Group IV: normal diet +18% alcohol (4g alcohol/kg body wt/day) + cigarette smoke-exposed.

Experimental rats from respective groups were administered with alcohol orally for a period of 30 days. Exposure to cigarette smoke was done by keeping rats in a bottomless metallic container, having two holes of 3 and 1.5 cm diameter, one on the either side, on the top of a polypropylene cage containing rats. A burning cigarette was introduced through one hole. Animals were exposed to cigarette

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smoke for 30 min daily for 30 days and at the end of experimental period, they were decapitated and the tissues — liver, lungs, heart and kidney were collected for various biochemical analyses.

MDA was assayed in terms of thiobarbituric acid reactive substances (TBARS), using 1,1,3,3-tetra-methoxypropane as the standard[18] and expressed as nmoles of MDA formed/mg protein. Hydroperoxides[19] and conjugated dienes[20] were estimated by the method described earlier. Catalase (CAT) was assayed[21] spectrophotometrically, following a decrease in absorbance at 243 nm and the specific activity was expressed as U/mg protein. Superoxide dismutase (SOD) was determined by the method reported previously[22]. One unit of enzyme was defined as the enzyme concentration required to inhibit OD at 560 nm of chromogen production of 50% in 1 min under the assay conditions and expressed as specific activity in U/mg protein.

Reduced glutathione (GSH) content was estimated[23] based on the reaction with 5,5'-dithiobis-(2-nitro-benzoic acid) and values were calculated from a standard plot of GSH. Concentration was expressed as nmoles/mg protein. Glutathione-S-transferase (GST) was estimated by the method[24] based on the rate of increase in conjugate formation between reduced glutathione (GSH) and 1-chloro-2, 4-dinitrobenzene (CDNB). Absorbance was measured at 340 nm for 5 min for an interval of one min. Concentration was expressed as µmoles/min/mg protein. Glutathione peroxidase (GPx) activity was determined[25] based on the degradation of hydrogen peroxide in the presence of GSH and the activity is expressed as U/mg protein. Protein in the sample was assayed by the method of Lowry *et al* [26].

Statistical analysis was carried out using students '*t*' test[27]. Values are expressed as mean ±SD.

Results and Discussion

The stress induced by alcohol, cigarette smoke and both in combination enhanced lipid peroxidation products, such as malondialdehyde, hydroperoxides and conjugated dienes (Table 1). The concentration of these products increased significantly in all the tissues, i.e., liver, heart, lungs and kidney in all the treated groups. While the level of significance of increase was at $P<0.05$ for lung tissues in alcohol-treated rats (group-II), the rest had a level of signi-

Table 1 — Concentration of malondialdehyde, hydroperoxides and conjugated dienes in the tissues of different groups of rats
[Values represent are mean ±SD of 6 rats in each group]

Group	Liver	Lungs	Heart	Kidney
Malondialdehyde (nmoles/mg protein)				
Group I	00.59±0.02	00.53±0.03	00.30±0.10	01.27±0.12
Group II	01.89±0.09*	00.99±0.02**	00.94±0.08*	02.98±0.09*
Group III	04.98±0.12*	03.97±0.09*	00.84±0.03*	02.67±0.10*
Group IV	06.02±0.19*	05.01±0.05*	02.99±0.07*	03.56±0.03*
Hydroperoxides (mmoles/100 g wet tissue)				
Group I	14.85±0.04	10.36±0.22	09.92±0.15	17.01±1.08
Group II	20.33±0.09*	12.68±0.26**	15.14±1.05*	24.05±0.99*
Group III	24.36±0.21*	30.06±0.39*	10.14±0.21	25.07±2.00*
Group IV	30.39±0.72*	40.29±0.17*	20.01±1.19*	30.07±0.93*
Conjugated dienes (mmoles/100 g wet tissue)				
Group I	46.09±3.02	10.36±0.21	08.99±0.36	17.01±.09
Group II	55.50±1.59*	12.01±2.00 **	18.18±0.58*	23.01±0.10*
Group III	66.98±3.70*	30.26±0.39 *	09.18±0.90	30.01±0.60 *
Group IV	78.01±3.01*	40.06±0.99*	22.80±0.99*	36.08±0.80*

*$P<0.001$ ** $P<0.05$

Group I — Control
Group II — alcohol-treated
Group III — smoke-exposed
Group IV — alcohol+smoke-exposed

ficance of *P*<0.001. It is also pertinent to note that the heart tissues in smoke-exposed rats did not show significant increase.

The SOD and CAT activities in all the tissues decreased significantly (*P*<0.001) in rats exposed to smoke and alcohol in combination (Table 2). No significant change was observed in the SOD/CAT activities in the heart of smoke-exposed rats. Similarly, the change in the SOD activity in the lungs of alcohol-treated rats was insignificant. However, SOD/CAT activities have shown a significant increase (*P*<0.001) in liver and lungs of alcohol-treated and smoke-exposed rats. In kidney tissues of smoke-exposed rats, these activities were different. While SOD activity showed a decrease, the CAT activity exhibited a significant increase. The SOD/CAT activity in liver, heart and kidney of alcohol-treated rats has shown a significant decrease (*P*<0.001). Lungs of alcohol-treated rats have also shown a decreased CAT activity (*P*<0.05).

Table 2 — Activities of superoxide dismutase, catalase, glutathione-S-tranferase, glutathione peroxidase and concentration of reduced glutathione in tissues of different groups of rats

[Values represent mean ± SD of six rats in each group]

	Liver	Lungs	Heart	Kidney
Superoxide dismutase[a] (U/mg protein)				
Group I	10.30±0.57	08.09±0.10	04.99±0.31	16.01±0.13
Group II	05.30±0.23*	07.88±0.29	02.01±0.01*	10.03±0.51*
Group III	14.20±0.93*	12.30±0.70*	04.69±0.18	11.01±0.31*
Group IV	04.33±0.92*	06.08 ±0.18*	01.99±0.19*	08.01±0.03*
Catalase[b] (U/mg protein)				
Group I	66.61±3.57	22.99±2.10	50.20±1.80	20.20±1.30
Group II	31.08±0.28*	21.08±0.99**	38.33±1.04*	14.36±0.92*
Group III	87.32±2.93*	39.98±3.90*	50.01±2.01	26.12±0.72*
Group IV	30.30±0.98*	15.18±2.11*	40.10±2.10*	10.38±0.63*
Glutathione-S-transferase[c] (µmoles/min/mg protein)				
Group I	01.02±0.06	01.90±0.07	-	00.90±0.08
Group II	03.82±0.07*	02.09±0.09	-	01.10±0.02
Group III	03.81±0.06*	03.89±0.03*	-	01.60±0.06**
Group IV	05.09±0.03*	07.00±0.09*	-	03.01±0.08*
Glutathione peroxidese (U/mg protein)				
Group I	00.18±0.06 0	00.14±0.07	00.21±0.09	00.26±0.05
Group II	00.47±0.09*	00.13±0.07	00.30±0.02**	00.25±0.02
Group III	00.59±0.07*	00.39±0.06*	00.20±0.07	00.27±0.01
Group IV	00.76±0.08*	00.60±0.07*	00.40±0.02*	00.49±0.03*
Reduced glutathione (nmoles/mg protein)				
Group I	10.38±0.19	07.81±0.50	16.08±0.09	06.02±0.21
Group II	06.08±0.29*	07.71±0.32	13.29±0.03*	04.29±0.30*
Group III	05.32±0.31*	04.98±0.32*	15.99±0.01	03.41±0.03*
Group IV	03.02±0.32*	04.02±0.18*	11.09±0.08*	03.42±0.09*

P<0.001; ***P*<0.05

[a]One unit of enzyme activity is defined as the amount of the enzyme required to inhibit OD at 560 nm of chromogen production by 50%/min

[b]One unit of enzyme activity is defined as the nmoles of H_2O_2 decomposed/min/mg protein

[c]One unit of enzyme activity is defined as one micromoles of CDNB conjugate formed/min/mg protein

- No data

Concentration of GSH was significantly decreased ($P<0.001$) (Table 2) in all the three groups, except heart of smoke-exposed and lungs of alcohol-treated rats. GST and GPx activities in liver, lungs and kidney have shown a significant increase ($P<0.001$) in all the treated groups but for alcohol-treated rats where the changes in lungs and kidney were insignificant (Table 2). Kidney tissues of smoke-exposed rats showed significant changes ($P<0.05$) in GST activity, while there was no change in GPx activity. GPx activity in heart tissue has shown significant changes in alcohol-treated rats ($P<0.05$) and also in alcohol and smoke-combination group ($P<0.001$), while there was no change in smoke-exposed group of rats. In the combined group, the GST in lungs was more than in liver, however the mechanism behind this increase is not clearly understood. In alcohol-treated and smoke-exposed rats, diet intake was reduced. The consumption was as follows: control, 15.6±3.1; alcohol-treated, 12.2±4.3; smoke-exposed, 13.5±2.6; and alcohol and smoke-combination group, 10.8±3.8 g diet/rat/day, respectively.

From the present study, it was observed that the stress induced by alcohol and smoke and both in combination enhanced lipid peroxidation. Ethanol is said to enhance the generation of oxygen free radicals during its oxidation in liver. The free radicals and oxidants in cigarette smoke are responsible for lipid peroxidation in different tissues of rats. The massive surface area makes lung a target organ for oxidative stress due to cigarette smoke[28]. Decreased activities of antioxidant enzymes, SOD and CAT, in alcohol-treated rats could be due to harmful effects of free radicals produced by administration of alcohol or direct effect of acetaldehyde formed from the oxidation of alcohol on these enzymes. SOD is responsible for dismutation of highly and potentially toxic compound superoxide radicals (O_2^-) to H_2O_2 and catalase converts it to H_2O and oxygen. A reduced activity of SOD and CAT may lower its cellular efficacy to detoxify these potentially active oxyradicals, thus leading to an increased level of lipid peroxidation products. An increase in the activity of GST and GPx could be a defense mechanism against the free radicals. The concentration of GSH is of clinical importance in tissue injury caused by toxic substances, which is a naturally occurring antioxidant. Binding of acetaldehyde, a metabolite of ethanol, with glutathione may contribute to reduction in the levels of GSH or it may be due to enhanced utilization of GSH by the antioxidant enzymes GST and GPx.

Cigarette smoke induces lipid peroxidation in all the tissues, except heart. It has been suggested that metabolism of toxic compounds, including free radicals, occurs mainly in liver and the metabolite from the liver diffuses into various extra hepatic tissues causing lipid peroxidation and cell injury. Smoke does not induce lipid peroxidation in heart. It has also been reported that the myocardium utilizes glucose in preference to fatty acids during stress for its energy requirement and the decreased utilization of fatty acids by the myocardial tissue may be one of the reasons for no change in lipid peroxide level in the heart[29]. Increased SOD and CAT activities due to smoke is suggestive possibly of an attempt made by the antioxidant defense to minimize the oxidative stress caused by increased free radical burden. Earlier, an increase in the activities of CAT and SOD and related enzymes of various tissues of rats exposed to cigarette smoke was also reported[30]. GSH is an important member of non-enzymatic defense system and disturbances in its levels may have serious implications. It is the substrate for GST and GPx. The activities of these enzymes are increased, thereby reducing the concentration of GSH. Increase in the activities of GST and GPx in the liver of alcohol-treated rats and liver and lungs of smoke-exposed rats might be a compensatory mechanism by the body against these oxidants. The decrease in the level of GSH in kidney of rats exposed to cigarette smoke was also reported earlier[31]. The decrease in glutathione was associated with the increased utilization of GSH by antioxidant enzymes GST and GPx. The increased activities of GST and GPx could be a defense mechanism against the free radicals.

In conclusion, combined exposure to alcohol and smoke enhanced oxidative stress, adversely affecting the antioxidant defense system and thus is likely to be more harmful than the effect of alcohol or smoke alone.

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