

N-Acetylcysteine Reduced the Effect of Ethanol on Antioxidant System in Rat Plasma and Brain Tissue

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Department of Biochemistry, ¹*Department of Internal Medicine,* ²*Experimental Animals Breeding and Research Laboratory, Cerrahpaşa Medical School,* ³*Department of Food Hygiene and Technology, School of Veterinary Medicine, Istanbul University, Istanbul, Turkey*

AYDIN, S., OZARAS, R., UZUN, H., BELCE, A., USLU, E., TAHAN, V., ALTUG, T., DUMEN, E. and SENTURK, H. *N-Acetylcysteine Reduced the Effect of Ethanol on Antioxidant System in Rat Plasma and Brain Tissue.* Tohoku J. Exp. Med., 2002, **198** (2), 71-77 — Chronic ethanol administration is able to induce an oxidative stress in the central nervous system. N-Acetylcysteine (NAC) has antioxidant properties; as a sulphhydryl donor, it contributes to the regeneration of glutathione and it acts through a direct reaction with hydroxyl radicals. In this study we investigated a possible beneficial effect of NAC on some of the free radical related parameters. Twenty four male Wistar rats were divided in to three groups and were given ethanol (Group 1), ethanol and NAC (Group 2) and isocaloric dextrose (Group 3). Ethanol and NAC were given intragastrically at doses of 6 g/kg/day and 1 g/kg/day, respectively. Our results show that chronic ethanol intake elicits statistically significant increase in MDA and NO levels and decrease in SOD and GSH levels in both plasma and brain ($p < 0.001$). GPx levels decreased in erythrocytes ($p < 0.001$). CAT activity showed significant decrease only in brain samples ($p < 0.001$). NAC administration effectively restores the above results to nearly normal levels. Therefore we suggest that reactive free radicals are, at least partly, involved in the ethanol-induced injury of brain cells and NAC mitigate the toxic effects of ethanol on the oxidant-antioxidant system of rat plasma and brain. — oxidative stress; n-acetylcysteine; ethanol; brain

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Received May 13, 2002; revision accepted for publication October 2, 2002.

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Ethanol promotes oxidative stress in several organs (Lieber 1994). Ethanol induced brain damage after long term exposure has been proven in neuronal and glial cell culture experiments in rats (Götz et al. 2001). Chronic ethanol exposure has recently been demonstrated to increase susceptibility of brain microsomes to iron-induced lipid peroxidation without modifying the glutathione (GSH) content, glutathione peroxidase (GPx) or glutathione disulfide reductase activity (GSSG-R) in whole brain (Omedeo et al. 1997). An increase in lipid peroxidation products and a decrease in cellular antioxidants have been recently suggested to confirm the involvement of oxidative stress in ethanol toxicity on rat peripheral nerves (Romero 1996; Morell et al. 1998). Although cerebellum is especially sensitive to oxidative stress probably due to its lowest concentration of α -tocopherol, other parts of the central nervous system can be affected by ethanol-induced lipid peroxidation (Nordmann et al. 1992). Increased lipid peroxidation was not observed in brain after ethanol administration to rats fed with vitamin-E supplemented diet (Nadiger et al. 1988).

N-Acetylcysteine (NAC) has antioxidant properties (Cuzzocrea et al. 2000; Farbiszewski et al. 2000; Rajakrishnan et al. 2000). As a sulphhydryl donor, it contributes to the regeneration of GSH (Harrison et al. 1991). NAC acts through a direct reaction with hydroxyl radicals (Aruoma et al. 1989). The challenge of an oxidative stress causes generation of prooxidant molecules and activation of the nuclear factor kappa B, leading to the expression of the inducible form of nitric oxide (iNOS) synthase. The produced NO reacts with superoxide and leads to formation of highly reactive nitrogen-containing species. NAC has been shown to inhibit NO production through iNOS (Bergamini et al. 2001) and inhibition of nuclear factor kappaB activation (Rota et al. 2002).

Recently, it has been shown that NAC

attenuates the toxic effects of methanol on the antioxidant system of the rat brain (Farbiszewski et al. 2000). NAC was also shown to successfully ameliorate oxidative stress and restore GSH content and GPx activity in rat peripheral nerves (Morell et al. 1998).

In this study, we aimed to study the effect of ethanol on oxidative and antioxidative parameters in rat plasma and brain tissue and to see whether co-administration of NAC attenuates oxidative parameters and/or improves antioxidative ones.

MATERIALS AND METHODS

Animals

Male Wistar-Albino rats weighing 220–250 g obtained from Istanbul University, Cerrahpasa Medical Faculty, Animal Breeding and Research Laboratory were kept in the same unit and fed chow (Eris Chow Industry, Istanbul, Turkey) ad libitum. All rats had free access to tap water. All experiments were done in accord with the standards in “The UFAW Handbook on the Care and Management of Laboratory Animals” (Poole 1999).

Twenty-four rats divided into three groups and were given ethanol (Group 1), ethanol and NAC (Group 2) and isocaloric dextrose (Group 3). In group 2, NAC was given 4 hours before alcohol in order to prevent chemical reactions, disturbances of absorption of both substance etc. NAC and alcohol were administered respectively 4 hours apart. Ethanol and NAC were given intragastrically at doses of 6 g/kg/day and 1 g/kg/day respectively. All rats were sacrificed at the end of one month under ether anesthesia. After exploration of the thorax, intracardiac blood was quickly obtained. Plasma alcohol levels were measured on the day the rats were sacrificed. GPx and GSH levels in erythrocytes were analyzed immediately. After decapitation, the brain was quickly removed, washed in cooled 0.15 M NaCl, kept on ice and subsequently blotted on filter paper. For the remaining studies plasma and brain

tissue samples were stored at -70°C .

Sample preparation

Brain samples were weighed and homogenized in 0.15 M NaCl for lipid peroxidation parameters and for the other studies, and homogenates of 20% were obtained. Tissue homogenates were sonicated two times at 30 seconds intervals. Homogenization and sonication were performed at 4°C . After sonication, homogenates for lipid peroxidation and the other parameters were centrifuged at 3000 rpm for 10 minutes and at 15 000 rpm for 15 minutes respectively. Protein levels were determined by the method of Lowry et al. (1951).

Biochemical assays

Lipoperoxidation was ascertained by the formation of malondialdehyde (MDA), which was estimated by the modified thiobarbituric acid method, described by Buege and Aust (1978). Cu-Zn superoxide dismutase (SOD) activity was determined by the method of Sun et al. (1988). Catalase activity (CAT) was

determined using Modified Aebi method (Yasminah et al. 1995). GSH concentration was determined according to the method of Beutler et al. (1963) using metaphosphoric acid for protein precipitation and 5' 5-dithiobis-2-nitrobenzoic acid for colour development. Erythrocyte and tissue glutathione concentrations were expressed as mg/g Hb, mg/g protein respectively. Hemoglobin concentration was determined by the cyanmethemoglobin method (Frankel et al. 1974). Plasma concentration of nitric oxide (NO) was estimated using commercially available colorimetric assay (Roche, Cat No. 1756281). Erythrocyte GPx levels were measured by using commercial kits (Randox-Ransel, Cat No. RS 505). Plasma alcohol level was measured by fluorescent polarizing immunoassay using commercial kits (Abbot TDx, Cat No. 378190100).

Statistical analysis

All results were expressed as mean \pm s.d.. Comparisons between the groups were performed by Kruskal-Wallis variance analysis and

TABLE 1. *The effect of NAC on oxidative and antioxidative parameters in rat plasma and brain tissue*

	Group 1 (Alcohol)	Group 2 (Alcohol+NAC)	Group 3 (Control)
MDA			
Plasma (nmol/ml)	1.84 \pm 0.14	0.91 \pm 0.14	0.94 \pm 0.11
Brain (nmol/g wet weight)	106.2 \pm 2.4	79.7 \pm 4.8	61.8 \pm 2.7
NO			
Plasma (μ mol/liter)	29.2 \pm 0.28	21.1 \pm 0.34	17.5 \pm 0.35
Brain (μ mol/mg protein)	0.548 \pm 0.015	0.503 \pm 0.012	0.463 \pm 0.012
SOD			
Plasma (U/ml)	11.08 \pm 1.13	17.92 \pm 0.81	19.68 \pm 1.76
Brain (U/mg protein)	8.28 \pm 0.33	9.48 \pm 0.32	10.8 \pm 0.35
CAT			
Plasma (U/ml)	168.8 \pm 7.2	184.5 \pm 7.2	182.4 \pm 13.8
Brain (U/mg protein)	2.01 \pm 0.27	2.81 \pm 0.17	2.82 \pm 0.25
GSH			
Erythrocyte (mg/g Hb)	3.56 \pm 0.18	3.87 \pm 0.13	3.98 \pm 0.18
Brain (mg/g protein)	0.94 \pm 0.18	1.34 \pm 0.21	1.58 \pm 0.14
GPx			
Erythrocyte (U/g Hb)	8.21 \pm 1.15	16.0 \pm 2.38	16.84 \pm 2.68

level of $p < 0.05$ was assumed to be statistically significant.

RESULTS

Table 1 shows values of the analysed parameters in the groups.

Plasma alcohol levels of Group 1 and 2 were comparable (207 ± 33 mg/100 ml and 192 ± 27 mg/100 ml).

Erythrocyte GPx level was lower in Group 1 (8.21 ± 1.15 U/g Hb) when compared to Group 2 (16.0 ± 2.38 U/g Hb) and Group 3 (16.84 ± 2.68 U/g Hb) ($p < 0.001$).

Erythrocyte GSH level was lower in Group 1 (3.56 ± 0.18 mg/g Hb) when compared to Group 2 (3.87 ± 0.13 mg/g Hb) and Group 3 (3.98 ± 0.18 mg/g Hb) ($p < 0.01$ and $p < 0.001$ respectively). Brain tissue GSH level was lower in Group 1 (0.94 ± 0.18 mg/g protein) when compared to Group 2 (1.34 ± 0.21 mg/g protein) and Group 3 (1.58 ± 0.14 mg/g protein) ($p < 0.001$).

Plasma SOD level was lower in Group 1 (11.08 ± 1.13 U/ml) when compared to Group 2 (17.92 ± 0.81 U/ml) and Group 3 (19.68 ± 1.76 U/ml) ($p < 0.001$). Brain tissue SOD level was lower in Group 1 (8.28 ± 0.33 U/mg protein) when compared to Group 2 (9.48 ± 0.32 U/mg protein) and Group 3 (10.8 ± 0.35 U/mg protein) ($p < 0.001$).

Plasma MDA level was significantly higher in Group 1 (1.84 ± 0.14 nmol/ml) than those in Group 2 (0.91 ± 0.14 nmol/ml) and Group 3 (0.94 ± 0.11 nmol/ml) ($p < 0.001$). Brain tissue MDA level was significantly higher in Group 1 (106.2 ± 2.4 nmol/g wet weight) than those in Group 2 (79.7 ± 4.8 nmol/g wet weight) and Group 3 (61.8 ± 2.7 nmol/g wet weight) ($p < 0.001$).

Plasma NO level was significantly higher in Group 1 (29.2 ± 0.28 μ mol/l) than those in Group 2 (21.1 ± 0.34 μ mol/l) and Group 3 (17.5 ± 0.35 μ mol/l) ($p < 0.001$). Brain tissue NO level was significantly higher in Group 1 (0.548 ± 0.015 μ mol/mg protein) than those in

Group 2 (0.503 ± 0.012 μ mol/mg protein) and Group 3 (0.463 ± 0.012 μ mol/mg protein) ($p < 0.001$).

Plasma CAT level in Group 1 (168.8 ± 7.2 U/ml) was similar to those in Group 2 (184.5 ± 7.2 U/ml) and Group 3 (182.4 ± 13.8 U/ml). Brain tissue CAT level was significantly lower in Group 1 (2.01 ± 0.27 U/mg protein) than those in Group 2 (2.81 ± 0.17 U/mg protein) and Group 3 (2.82 ± 0.25 U/mg protein) ($p < 0.001$).

DISCUSSION

Brain and nervous system are more vulnerable to ethanol-induced oxidative damage than the liver. This vulnerability is due to the intrinsically low activities of the CAT, GPx and SOD (Farbiszewski et al. 2000). The brain has low levels of both proteins and also antioxidative enzyme proteins (Harris 1992). It has high rate of oxygen consumption, higher percentage of polyunsaturated fatty acids than the plasma and the essentially non-regenerative nature (Farbiszewski et al. 2000). NO has been also suggested to have a role in the formation of the injury-initiating oxidative stress (White et al. 1993). NO reacts with superoxide to form the very toxic compound peroxynitrite (Cuzzocrea et al. 2000). Peroxynitrite initiates lipid peroxidation, inactivates enzymes such as mitochondrial respiratory enzymes and membrane pumps and also depletes glutathione (Cuzzocrea et al. 2000). GSH and GSH-related enzymes have important roles in the protection of the brain (Farbiszewski et al. 2000). The increase in antioxidants after administration of protectors attenuates the toxicity of alcohol in the cells and decreases their vulnerability to injury (Lieber 1994).

Ethanol oxidation in rat brain has been reported to involve CAT. Aragon et al. (1989) suggested that H_2O_2 is generated in the rat brain and acetaldehyde formation through CAT may be responsible for some of the psychopharmacological actions of ethanol (Cohen et al. 1980; Aragon et al. 1991). In our study, while

plasma CAT levels were not different among the groups, it was significantly lower in brain tissue of the ethanol group. This result can be explained by the previous findings that ethanol decreases mitochondrial CAT activity by nearly 70-80% (Reddy et al. 1999).

Ischemic brain injury has been shown to increase MDA levels in gerbils and NAC successfully improved brain injury in this setting (Cuzzocrea et al. 2000). It is demonstrated that NAC inhibits NO production: In gerbils, it has been seen an increase in the plasma levels of nitrite/nitrate at 4 hours after reperfusion compared with plasma collected from sham animals and NAC treatment before and after reperfusion reduced the nitrite/nitrate formation significantly. This inhibition was thought to be due to removal of superoxide anion and potentiation of feedback inhibition on NO synthase by NO via inactivation of its heme centre (Cuzzocrea et al. 2000).

In this study we selected NAC as a safe and effective antioxidant (Prescott et al. 1979; Howard et al. 1987). It can react with membrane protein sulfhydryl groups and protect stabilizing membrane lipid-protein structure against oxidation. It replaces intracellular reduced glutathione (Bruck et al. 1990).

NAC has been reported to enhance hippocampal neuronal survival suggesting that the scavenging of free radicals by NAC is the most likely mechanism of NAC action (Kunkey et al. 1995). In a recent study, Farbiszewski et al. (2000) studied antioxidant roles of NAC and a trolox derivative in rat brain. NAC was administered in a dose of 150 mg/kg intraperitoneally and subsequently methanol was given. NAC was shown to increase the activities of SOD, GPx and GSSG-R better than the trolox derivative.

Also, Cuzzocrea et al. (2000) studied the effects of NAC on ischemic brain injury. NAC, in a dose of 20 mg/kg intraperitoneally was given 30 minutes before reperfusion and 1, 2 and 6 hours after reperfusion in gerbils. They

found that NAC increased survival and reduced hyperactivity linked to neurodegeneration induced by cerebral ischemia and reperfusion; histological studies showed reduction of neuronal loss. They concluded that NAC improves brain injury induced by transient cerebral ischemia.

NAC has been shown to normalize MDA content and to restore GSH content and GPx activity in peripheral nerves of chronic ethanol-fed rats (Morell et al. 1998). In this study NAC was given in liquid diets and seemed to be a better protector in oxidative stress than S-adenosyl methionine.

In various brain areas, chronic ethanol administration was shown to increase heat-shock proteins, and this increase correlated with a marked depletion of bound thiols and with a decreased susceptibility to lipid peroxidation in rats (Calabrese et al. 1998). Lower levels of heat-shock proteins were found in cortex and cerebellum and were associated to decreases in SOD and CAT enzyme activities.

In our study, NO activities in both plasma and brain tissue in NAC group were lower than alcohol group but still higher than control group. The same was true for brain MDA level but plasma MDA level in NAC group was comparable to control and both being lower than alcohol group.

In conclusion, alcohol both induces oxidative stress and also reduces antioxidant activity of various tissues including brain. We have demonstrated that co-administration of an antioxidant, NAC, both attenuates increased oxidative stress parameters such as MDA, NO and restores diminished anti-oxidant enzymes and substances such as GPx, CAT, SOD and GSH; thus protects central nervous system from ethanol-induced lipid peroxidation. This study demonstrated that alcohol administration leads to the changes in both oxidative and anti-oxidative parameters that can be measurable in serum and brain tissue. Concurrent use of a powerful but meanwhile safe antioxidant

substance can attenuate these changes. Randomized and controlled clinical studies may define whether these changes can be seen also in alcoholic patients.

Acknowledgments

We thank to Bilim Pharmaceutical Company for their financial support.

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