Polish Journal of Pharmacology
Pol. J. Pharmacol., 2003, 55, 1037–1043
ISSN 1230-6002

TAURINE PREVENTS ACRYLONITRILE-INDUCED OXIDATIVE STRESS IN RAT BRAIN

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Taurine prevents acrylonitrile-induced oxidative stress in rat brain. K. MAHALAKSHMI, G. PUSHPAKIRAN, C.V. ANURADHA. Pol. J. Pharmacol., 2003, 55, 1037–1043.

Acrylonitrile (ACN) is a volatile, toxic liquid used as a monomer in the manufacture of synthetic rubber, styrene plastics, acrylic fiber and adhesives. ACN is a potent neurotoxin and a carcinogen, which produces tumors in rats, particularly gliomas of the brain. A role for free radical-mediated lipid peroxidation in the toxicity of ACN has been suggested. We examined the ability of taurine, an antioxidant amino acid, to attenuate ACN-induced alterations in lipid peroxidation, cellular DNA fragmentation, GSH, vitamin C and vitamin E levels in blood and brain of rats. Rats were administered with ACN at a concentration of 100 ppm in drinking water and sacrificed after 14 and 28 days. The level of lipid peroxidation and the enzymatic and non-enzymatic antioxidants were assayed. The obtained data were compared with those obtained from ACN rats co-treated with taurine for 14 and 28 days. It was observed that taurine treatment counteracted the oxidative stress induced by ACN by reducing the levels of peroxidation, and enhancing the activities of enzymatic and non-enzymatic antioxidants.

Key words: *acrylonitrile, antioxidants, DNA fragmentation, lipid peroxidation, taurine*

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INTRODUCTION

Acrylonitrile (CH_2=CH–C≡N, ACN) is a volatile, colorless, flammable liquid with a characteristic odour. It is used as an intermediate in the production of acrylic fibers, resins and rubbers. Human exposure to ACN could potentially occur during the manufacturing process, end product usage and transportation. Further, exposure can also be possible in the general population through cigarette smoke and *via* contamination of drinking water [5]. Chronic exposure of rats to ACN has been shown to produce a dose related increase in glial cell tumors (astrocytomas) [4]. Previous studies show that ACN exposure is associated with the induction of oxidative stress. Increased peroxidation of lipids, oxidative damage to DNA and deficiency in antioxidant protection in rat brain following *in vivo* ACN exposure and in rat glial cells *in vitro* have been observed [15, 18].

Taurine (2-aminoethanesulfonic acid) is the major intracellular free β-amino acid present in most mammalian tissues [6]. Taurine is involved in growth and development. It possesses a number of cytoprotective properties through its actions as an antioxidant [23], osmoregulator, intracellular calcium flux regulator and neuromodulator [12]. The beneficial effect of taurine as an antioxidant in biological system has been attributed to its ability to stabilize biological membrane, scavenge reactive oxygen species [38] and reduce the production of MDA, an end product of lipid peroxidation. Previously, we showed that taurine positively influenced antioxidant capacity and minimized peroxidative damage in plasma and tissues of insulin resistant rats [24].

No previous studies are available on the effect of taurine on ACN-induced toxicity in rats. We now report the effect of taurine on the lipid peroxidation status and the activities of enzymatic antioxidants: superoxide dismutase, catalase, glutathione peroxidase and non-enzymatic antioxidants: GSH, vitamin C and E in blood and brain of rats treated with ACN. In addition, the effect of taurine on DNA fragmentation induced by ACN was determined.

MATERIALS and METHODS

Male adult Wistar rats (170–190 g) were obtained from the Central Animal House, Rajah Muthiah Medical College, Annamalai University. They were housed in an animal room under controlled conditions on a 12 h light/12 h dark cycle. They received a standard pellet diet (Kamadenu Agencies, Bangalore, India) and water *ad libitum*.

The study protocol using animals was approved by the Institutional Animal Ethical Committee (IAEC) of Rajah Muthiah Medical College, Annamalai University. After acclimatization, the animals were divided into the following groups consisting of 6 rats each.

Group 1. Control animals (CON) received commercial diet and tap water *ad libitum*.
Group 2. Animals received ACN for 14 days.
Group 3. Animals received ACN for 28 days.
Group 4. Animals received ACN and taurine for 14 days.
Group 5. Animals received ACN and taurine for 28 days.
Group 6. Control animals received only taurine for 28 days.

ACN was administered in drinking water at a concentration of 100 ppm. Taurine (10 g/kg diet) was supplied in the diet. Food and water were provided *ad libitum*.

The animals were maintained in their respective groups for 28 days. Food and fluid intake and body weights were measured weekly. At the end of the experimental period, the animals were anesthetized using ether and sacrificed by cervical decapitation. Blood was collected from the jugular vein using heparin as the anticoagulant and centrifuged at 2000 g for 20 min to prepare plasma. The red blood cells (RBC) were washed thrice with physiological saline. An aliquot of 0.5 ml of washed RBC was lysed with 4.5 ml of ice-cold 0.01 M Tris HCl buffer, pH 7.4 to prepare hemolysate.

Brain tissues were cleared of adhering fat, weighed accurately and cut into fragments and immediately homogenized to a known volume of 0.1 M Tris Hcl buffer, pH 7.4 using a Potter – Elvehijam homogenizer with a teflon pestle. Assays were carried out in plasma, hemolysate and tissue homogenates.

Biochemical analysis

The concentration of thiobarbituric acid reactive substances (TBARS) were estimated in the plasma and brain by the method of Niehaus and Samuelsson [26] using 1,1',3,3'-tetramethoxypropane as the standard. Lipid hydroperoxides in plas-

ma and methanol-extracted brain homogenates by the FOX assay described by Jiang et al. [16]. For this assay, plasma or the tissue lipid extract was treated with FOX reagent (88 mg of butylated hydroxytoluene (BHT), 7.6 mg of xylenol orange and 9.8 mg of ammonium iron (II) sulphate in 90 ml of methanol and 10 ml of H_2SO_4). The color formed was read at 560 nm after 30 min. The amount of hydroperoxides produced was calculated using the molar extinction coefficient of 4.6×10^4 M^{-1} cm^{-1}. The values are expressed as μmol/l in plasma and nmol/mg of protein in brain. The non protein-thiol (NP-SH) content was measured in plasma and brain by the method of Ellman [8]. Briefly, plasma or tissue homogenate was treated with TCA solution (5% for plasma and 50% for tissue). The thiol content was determined in the supernatants by reaction with dithionitrobenzoic acid (DTNB). The values are expressed as mg/dl in plasma and μg/mg of protein in brain.

Superoxide dismutase (SOD) activity in the hemolysate and brain was assayed by the method of Kakkar et al. [17] based on the inhibition of formation of NADH – phenazine methosulfate-nitroblue tetrazolium complex. One unit of SOD corresponds to the amount of enzyme causing 50% reduction of nitro blue tetrazolium/min/mg of Hb or protein. Catalase (CAT) was assayed by the method of Sinha [35] by quantitating the H_2O_2 consumed after the enzymatic reaction. Dichromate in acetic acid was used as the coloring agent. The activity of catalase is expressed as μmol H_2O_2 consumed/min/mg of protein. GPx activity was assayed in hemolysate and brain by the method of Rotruck et al. [32]. A known amount of enzyme preparation was allowed to react with hydrogen peroxide (H_2O_2) and glutathione (GSH) for a specified time period. The GSH content remaining after the reaction was measured by reaction with DTNB. The GPx activity is expressed as μmoles of GSH oxidized/min/mg of protein. Glutathione-S-transferase (GST) in the hemolysate and brain was assayed by the method of Habig [11] by following the increase in absorbance at 340 nm using 1-chloro-2,4-dinitrochlorobenzene (CDNB) as substrate. The GST activity is expressed as mmol of glutathione-chlorodinitrobenzene conjugate formed/min/mg of protein.

The concentration of ascorbic acid was determined in plasma and brain by the method of Omaye et al. [27], and vitamin E was measured by the method of Baker and Frank [3]. The values are expressed as μg/mg of protein. The extent of DNA fragmentation was determined by the method adapted from that of Lin et al. [20]. Brain tissue homogenate was treated with 0.01 M Tris buffer pH 8.0, 1 mM EDTA and 0.5% triton X-100 and centrifuged. Both supernatant and pellet were precipitated with 12.5% TCA. Quantitative analysis of DNA was carried out by diphenylamine reaction. The percentage of fragmentation was calculated from the ratio of DNA in the supernatant to the total DNA. Hemoglobin content was analyzed in the hemolysate by the method of Drabkin and Austin [7]. Protein content in the tissue was estimated by the technique of Lowry et al. [21].

Statistical analysis

Values are given as means ± SD. The difference between groups were analyzed using ANOVA followed by Duncan's Multiple Range Test (DMRT). The level of significance was set at $p < 0.05$.

RESULTS

The food intake of animals did not vary between the experimented groups. Water consumption was measured in each treatment group daily during the experimental period. The average uptake of ACN over the treatment period was 8–10 mg/kg/day for the rats, which received 100 ppm of ACN and that of taurine was 600–800 mg/kg/day. No statistically significant differences in body weights were seen between the treatment groups after 14 and 28 days of treatment. No statistical differences in brain weight were observed in any of the treatment group (Tab. 1).

Table 2 gives the levels of lipid peroxidation products, such as TBARS and hydroperoxides in plasma and brain and percentage of DNA fragmentation in the brain. There was a significant increase in TBARS (12% in plasma and 23% in brain) and hydroperoxides (20% in plasma and brain) of ACN-treated rats. The percentage of DNA fragmentation was significantly increased in plasma and brain of ACN-treated rats (Groups 2 and 3). The levels were near normal in taurine supplemented ACN-treated and control rats (Groups 4, 5 and 6).

Table 3 gives the activities of enzymatic antioxidants in the hemolysate and brain of control and experimental rats. Rats treated only with ACN showed significant reduction in the activities of

Table 1. Body weights of control and experimental animals (values are expressed as means ± SD; n = 6)

	CON	ACN14	ACN28	ACN + TAU14	ACN + TAU28	CON + TAU
Body weight (g)						
Initial	182.3 ± 1.9	182.0 ± 4.6	181.2 ± 6.9	183.7 ± 6.7	183.5 ± 0.8	182.0 ± 2.9
Final	245.8 ± 5.3	244.0 ± 7.2	243.7 ± 6.5	245.7 ± 4.5	242.5 ± 4.5	245.8 ± 4.1
Organ weight (g)						
Liver	6.74 ± 0.11	6.68 ± 0.02	6.65 ± 0.05	6.70 ± 0.05	6.70 ± 0.05	6.74 ± 0.07
Brain	1.70 ± 0.03	1.67 ± 0.04	1.66 ± 0.08	1.65 ± 0.02	1.66 ± 0.02	1.69 ± 0.03

CON – control; ACN14 – ACN-treated animals (14 days); ACN28 – ACN-treated animals (28 days); TAU – taurine

Table 2. Lipid peroxidation products in plasma and brain of control and experimental animals (values are expressed as means ± SD; n = 6)

	CON	ACN14	ACN28	ACN + TAU14	ACN + TAU28	CON + TAU
TBARS						
Plasma (μmol/l)	2.94 ± 0. 028	3.34 ± 0.064*	3.33 ± 0.042*	2.93 ± 0.10♦	2.95 ± 0.039#	2.94 ± 0.043
Brain (nmol/mg of protein)	1.78 ± 0.052	2.31 ± 0.029*	2.36 ± 0.074*	1.80 ± 0.035♦	1.76 ± 0.045#	1.76 ± 0.036
Lipid hydroperoxides						
Plasma (μmol/l)	0.42 ± 0.031	0.58 ± 0.024*	0.61 ± 0.038*	0.45 ± 0.025♦	0.44 ± 0.030#	0.43 ± 0.021
Brain (nmol/mg of protein)	1.76 ± 0.033	2.30 ± 0.088*	2.33 ± 0.039*	1.75 ± 0.052♦	1.71 ± 0.077#	1.71 ± 0.020
DNA fragmentation (%)	8.83 ± 2.04	14.16 ± 2.48*	16 ± 2.09*	11.50 ± 1.87♦	10.50 ± 1.87#	9.50 ± 1.870

CON – control; ACN14 – ACN-treated animals (14 days); ACN28 – ACN-treated animals (28 days); TAU – taurine. * Significantly different when compared with control ($p < 0.05$); ♦ significantly different when compared with ACN 14 days ($p < 0.05$); # significantly different when compared with ACN 28 days ($p < 0.05$)

Table 3. Levels of enzymatic antioxidants in hemolysate and brain of control and experimental rats (values are expressed as means ± SD; n = 6)

	CON	ACN14	ACN28	ACN + TAU14	ACN + TAU28	CON + TAU
SOD (Units)						
Hemolysate	53.97 ± 3.70	27.29 ± 1.82*	26.57 ± 1.21*	48.46 ± 2.31♦	51.46 ± 4.70#	51.29 ± 5.88
Brain	8.33 ± 0.31	5.89 ± 0.045*	5.83 ± 0.42*	7.91 ± 0.11♦	8.10 ± 0.06#	8.08 ± 0.06
CAT (Units)						
Hemolysate	53.97 ± 3.70	27.29 ± 1.82*	26.57 ± 1.21*	48.46 ± 2.31♦	51.46 ± 4.70#	51.29 ± 5.88
Brain	3.22 ± 0.31	1.21 ± 0.11*	1.09 ± 0.05*	2.99 ± 0.25♦	3.02 ± 0.29#	3.21 ± 0.39
GPx (Units)						
Hemolysate	19.69 ± 0.87	12.07 ± 0.91*	11.12 ± 1.12*	18.79 ± 0.76♦	17.75 ± 1.19#	19.12 ± 1.84
Brain	3.36 ± 0.32	1.37 ± 0.14*	1.27 ± 0.24*	3.09 ± 0.54♦	3.27 ± 0.56#	3.33 ± 0.13
GST (Units)						
Hemolysate	6.18 ± 0.13	4.89 ± 0.09*	4.73 ± 0.18*	6.13 ± 0.28♦	6.17 ± 0.13#	6.30 ± 0.07
Brain	5.68 ± 0.10	4.65 ± 0.17*	4.52 ± 0.13*	5.56 ± 0.31♦	5.59 ± 0.24#	5.59 ± 0.10

CON – control; ACN14 – ACN-treated animals (14 days); ACN28 – ACN-treated animals (28 days); TAU – taurine; SOD Units – 50% nitroblue tetrazolium reduction/min/mg of Hb or protein; CAT Units – μmol H_2O_2 consumed/min/mg of protein; GPx Units – μmoles of GSH oxidized/min/mg of protein; GST Units – mmol of glutathione-chlorodinitrobenzene conjugate formed /min/mg of protein. * Significantly different when compared with control ($p < 0.05$); ♦ significantly different when compared with ACN 14 days ($p < 0.05$); # significantly different when compared with ACN 28 days ($p < 0.05$)

Table 4. Levels of non-enzymatic antioxidants in plasma and brain of control and experimental animals (values are expressed as means ± SD; n = 6)

	CON	ACN14	ACN28	ACN + TAU14	ACN + TAU28	CON + TAU
NP-SH						
Plasma (mg/dl)	33.58 ± 2.7	17.50 ± 0.1*	15.39 ± 0.23*	31.70 ± 3.1$^{\blacklozenge}$	30.34 ± 5.53$^{\#}$	32.44 ± 0.24
Brain (μg/mg of protein)	4.62 ± 0.7	3.34 ± 0.56*	2.86 ± 0.34*	4.58 ± 0.58$^{\blacklozenge}$	4.54 ± 0.61$^{\#}$	4.80 ± 0.56
Ascorbic acid						
Plasma (μg/mg of protein)	2.54 ± 0.28	1.75 ± 0.19*	1.71 ± 0.21*	2.44 ± 0.26$^{\blacklozenge}$	2.32 ± 0.25$^{\#}$	2.61 ± 0.19
Brain (μg/mg of protein)	0.26 ± 0.018	0.17 ± 0.007*	0.15 ± 0.015*	0.24 ± 0.027$^{\blacklozenge}$	0.25 ± 0.023$^{\#}$	0.26 ± 0.019
α-tocopherol						
Plasma (μg/mg of protein)	1.32 ± 0.024	0.71 ± 0.023*	0.70 ± 0.022*	1.29 ± 0.016$^{\blacklozenge}$	1.28 ± 0.038$^{\#}$	1.30 ± 0.033
Brain (μg/mg of protein)	0.27 ± 0.024	0.17 ± 0.018*	0.16 ± 0.021*	0.30 ± 0.028$^{\blacklozenge}$	0.27 ± 0.021$^{\#}$	0.28 ± 0.037

CON – control; ACN14 – ACN-treated animals (14 days);ACN28 – ACN-treated animals (28 days); TAU – taurine. * Significantly different when compared with control ($p < 0.05$); $^{\blacklozenge}$ significantly different when compared with ACN 14 days ($p < 0.05$); $^{\#}$ significantly different when compared with ACN 28 days ($p < 0.05$)

these enzymes in plasma. SOD activity was reduced by about 50% in plasma and 30% in brain, GPx was lower by about 60% in brain while GST registered a decline by about 20% in plasma and brain. Taurine supplementation restored the activities of these enzymes.

Table 4 shows the levels of non-enzymatic antioxidants in plasma and brain. The levels of these antioxidants were significantly lowered. NP-SH was reduced by about 45% in plasma and 28% in brain, ascorbic acid was reduced by 30% in plasma and brain while vitamin E was reduced by 40% in both plasma and brain of ACN-treated rats as compared to that of control rats. The levels were higher in the control and ACN-treated rats supplemented with taurine than in rats treated only with ACN.

DISCUSSION

The increased levels of lipid peroxidation end products in plasma and brain and damage to brain DNA, a critical cellular macromolecule were observed in the present study in rats after 14 and 28 days of treatment with ACN. This is in agreement with the findings of other authors. Induction of lipid peroxidation in the rat brain by ACN [15] and significant increases in the concentration of 8-hydroxydeoxyguanosine (8OHdG), a product of DNA oxidation, were observed in the brain. Several studies have indicated the role of oxidative stress in the toxicity of ACN. For instance, the major pathway of ACN elimination is its conjugation with GSH to form mercapturic acid through the GST [10]. By depleting GSH, ACN may decrease the antioxidant levels of the cells leading to an overall increase in intercellular reactive oxygen species (ROS) and oxidative damage. Metabolism of ACN results in the production of cyanide. Cyanide has been shown to induce oxidative stress (lipid peroxidation) in the brain of acutely treated mice and cell lines by inhibiting mitochondrial respiratory chain, CAT and glutathione peroxidase [19, 22].

The results of our study show that both enzymatic and non-enzymatic antioxidant defense systems are impaired in ACN-treated rats. The effects of ACN on the suppression of activities of SOD and CAT may be related to the toxicity of ACN. GPx and GST are glutathione-dependent intracellular enzymatic antioxidants. GPx is responsible for the removal of ROS, such as peroxides, while GST is essential for conjugation. ACN may decrease the activities of these enzymes by defective synthesis or inactivation by binding. ACN is shown to bind covalently to rGSTM1, a specific subunit of GST in rat liver [25].

The non-enzymatic free radical scavengers, GSH, vitamin E and vitamin C exist in their interconvertible forms and participate in the detoxification of the reactive oxygen species. GSH participates in enzymatic reduction of membrane hydroperoxy-phospholipids and prevents the formation of secondary alkoxyl radicals when organic peroxides are homolyzed [33]. Binding of ACN to these antioxidants, especially GSH, results in the induc-

tion of oxidative stress and impaired regeneration of other antioxidants. Studies with ^{14}C ACN have shown that ACN covalently binds with sulfhydryl groups of protein [1] and to tissue macromolecules and nucleic acids [30]. This explains the reduction in GSH content of tissues. Depletion of GSH in cells increases their susceptibility to oxidative damage.

Administration of taurine significantly attenuated ACN-induced lipid peroxidation and prevented DNA fragmentation in rat brain. Taurine attenuates lipid peroxidation either by scavenging and inactivating H_2O_2 and hydroxyl radicals or by binding Fe^{2+} like a chelator [36]. Taurine also scavenges the free radicals formed through various reactions catalyzed by Fe^{2+} [9]. Taurine may also terminate lipid peroxidation by inducing enzymatic and non-enzymatic antioxidants.

The antioxidant vitamin E has been shown to prevent ACN-induced increases of hepatic enzymes in serum [13], lipid peroxidation in liver microsomes [34] and decrease in brain γ-aminobutyric acid (GABA) and glutamate decarboxylase in rats treated with ACN [2]. ACN-induced morphological transformation and oxidized DNA in Syrian hamster embryonic cells was blocked by vitamin E co-treatment [40]. The increased ethane formation by ACN was prevented by vitamin E co-treatment [14].

Vitamin E protects membrane lipids against oxygen damage. It is now well established that taurine also has such antioxidant properties in the tissue and is a membrane stabilizer [28]. Protection of the liver against lipid peroxidation and membrane disintegration by taurine during rat hepatocarcinogenesis has been reported [39]. Taurine may exert its antioxidative action by conjugation with different types of α-tocopherols. Taurine may potentially convert water-insoluble vitamin E into water-soluble forms [29].

Taurine serves to counteract the deleterious effects of other toxic chemicals, such as ammonia-induced excitotoxicity [41], acetaminophen [37] and ethanol [31]. It is possible that taurine may modify factors underlying susceptibility to toxic chemicals.

The present findings show that taurine treatment can attenuate lipid peroxidation in the rat brain induced by ACN by up-regulating the activities of enzymatic antioxidants and by replenishing non-enzymatic antioxidants. These results indicate that taurine merits further investigations as a potential supplement for smokers and for those prone to occupational exposure to ACN.

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Received: August 18, 2003; in revised form: November 3, 2003.