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EFFECT OF METHACRYLONITRILE ON MEMBRANE BOUND ENZYMES OF RAT BRAIN

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Abstract : Methacrylonitrile (MeAN) is a plastic monomer. Its effect on membrane bound enzymes like Na^+K^+-ATPase, Ca^{2+}-ATPase, Mg^{2+}-ATPase, NADH dehydrogenase, alkaline phosphatase (ALP) and various elements like sodium (Na^+), potassium (K^+), and calcium (Ca^{2+}) in rat brain were studied. Administration of 50 mg/kg body weight/day (0.25 LD_{50}) and 100 mg/kg body weight/day (0.5 LD_{50}) by gavage to rats for 7 days resulted in a significant decrease in activities of Na^+K^+-ATPase, Ca^{2+}-ATPase, Mg^{2+}-ATPase, and NADH dehydrogenase. A significant reduction in calcium content, potassium content and a significant increase in sodium content and alkaline phosphatase activity in MeAN treated animals were observed. Inhibition of membrane bound enzymes occurred due to either direct effect of MeAN or indirect effect of changes in ionic homeostasis in MeAN treated animals.

Key words : methacrylonitrile membrane bound enzyme brain

INTRODUCTION

Methacrylonitrile (MeAN) is an aliphatic nitrile, which is produced in large quantities and it is used as a replacement for acrylonitrile (AN) in the production of plastics, elastomers, coatings and in the manufacture of carbonated beverage containers. MeAN has been identified as a component of the main stream smoke of unfiltered cigarettes made from air-cured, flue-cured, or a blend of these tobaccos (1). MeAN has been shown to be toxic in rats, dogs and rabbits by dermal, inhalation, intraperitoneal, ocular and gavage routes (2). In addition, MeAN depletes glutathione *in vivo* and *in vitro* (3). MeAN is thought to undergo metabolism via pathways qualitatively similar to that of AN (4). Methacrylonitrile metabolism leads to the liberation of cyanide ions in the blood (5). In comparison to other organs, blood, liver and

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brain contained the highest amounts of cyanide (6). The toxic actions of MeAN were attributed to the inactivation of cytochrome oxidase leading to disturbances in tissue respiration (7). MeAN administration, in rat, mouse and gerbil developed several central nervous system abnormalities, including depression, ataxia, asphyxia, irregular breathing, trembling, and convulsion (8). Similar pathological signs were observed in rats and mice treated with potassium cyanide (9). Literature on the toxic influence of MeAN on central nervous system is completely lacking. We therefore undertook the present study to characterize the toxic effects of MeAN on membrane bound enzymes of rat brain.

MATERIAL AND METHODS

Wistar strain male albino rats weighing about 120–150 g were used for the study. Sublethal doses (50 mg/kg body weight/day (0.25 LD_{50}) and 100 mg/kg body weight/day (0.5 LD_{50}) for 7 days of methacrylonitrile (Fluka Cheme AG, CH-9470 Buchs, Switzerland) in sunflower oil were administered orally to experimental animals (Group 2 and Group 3). The dosages were chosen according to the report (10). Control animals (Group 1) were given sunflower oil only. After the experimental period animals were sacrificed after an over night fast by cutting jugular vein. Brain was dissected and washed in ice-cold saline and homogenized at 4°C (10% w/v) in 0.1 M Tris HCl, pH 7.4. Homogenate was centrifuged at 2000 × g for 10 min and supernatant was used for the assays. Total protein was determined by the method of Lowry et al (11). Na^+K^+-ATPase activity was assayed according to a method described previously (12). Ca^{2+}-ATPase activity was estimated according to the method (13). Mg^{2+}-ATPase activity was measured according to the method (14). Alkaline phosphatase activity (ALP) was assayed by measuring the phenol liberated from disodium phenylphosphate (DSPP) by the colour reaction with Folin's reagent in the presence of an alkali according to the method (15). NADH dehydrogenase (NADH-DH) was assayed according to the method (16). One gram of tissue was placed in a Kjeldalhl's flask 2.5 ml of de-ionized water and one ml of 1:1 mixture of concentrated nitric acid and 70% perchloric acid were added. The samples were digested on a sand bath till the solutions became clear. The digest was made up to 50 ml with de-ionized water and mixed thoroughly. Sodium, potassium and calcium concentrations were estimated by using atomic absorption spectrophotometer.

For histological study portion of brain from the control and MeAN treated rats were gently rinsed with physiological saline solution (0.9% NaCl) to remove blood and debris adhering to tissues. The tissues were then fixed in 10% formalin for 24 hrs. After dehydration through a graded series of alcohols, the tissues were cleaned in methyl benzoate, embedded in paraffin wax. Sections were cut at 5 µm thickness and stained with hematoxylin and eosin.

Statistical analyses were done by Student's test. A 'P' value less than 0.05 were considered as significant. The ethical committee of the institute has given the necessary ethical clearance for this work.

RESULTS

The data after statistical analyses are presented in Tables I and II. Table I shows the activities of Na^+K^+-ATPase, Ca^{2+}-ATPase, Mg^{2+}-ATPase and NADH dehydrogenase were significantly ($P<0.001$) reduced and a significant ($P<0.001$) increase in alkaline phosphatase (ALP) in MeAN treated rats compared with control group (Group I). Table II shows the level of sodium (Na^+) was significantly increased ($P<0.001$), whereas those of calcium (Ca^{2+}) and potassium (K^+) were significantly reduced ($P<0.001$) in MeAN treated rats as compared to control group (Group 1).

DISCUSSION

The observed decrease in the activities of Na^+K^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase in MeAN treated rats (Table I) may be due to the change in ionic homeostasis and may also be due to ATP depletion (17). Na^+K^+-ATPase uses chemical energy provided by ATP to trans-locate Na^+ and K^+, and build up an electrochemical potential gradient across the plasma membrane (18). Since MeAN is a cyanide releasing compound, which is a potent ATP depletor, would have decreased the activity of Na^+K^+-ATPase. Inhibition of Na^+K^+-ATPase by MeAN in RBC membrane has been reported earlier (19).

TABLE I: Activities of Na^+K^+-ATPase, Ca^{2+}-ATPase, Mg^{2+}-ATPase NADH dehydrogenase and Alkaline phosphatase in whole brain of control and MeAN treated rats.

Parameters	*Group 1 (Control)*	*Group 2 (50 mg/kg body weight/day)*	*Group 3 (100 mg/kg body weight/day)*
Na^+K^+-ATPase (µ moles of Pi liberated/min/mg protein)	0.56±0.014	0.40±0.072***	0.34±0.022***
Ca^{2+}-ATPase (µ moles of Pi liberated/min/mg protein)	0.57±0.087	0.37±0.028***	0.29±0.037***
Mg^{2+}-ATPase (µ moles of Pi liberated/min/mg protein)	0.65±0.123	0.60±0.016***	0.32±0.025***
NADH dehydrogenase (µ moles of $K_3Fe(CN)_6$ reduced/min/mg protein)	0.09±0.003	0.017±0.002***	0.06±0.001***
Alkaline phosphatase (µ moles of phenol liberated/min/mg protein)	87.1±7.10	140.0±15.8***	150.0±15.2***

Values are expressed as mean±S.D. for six rats in each group, ***P<0.001.

TABLE II: Levels of sodium, potassium, and calcium in control and MeAN treated rat brain.

Parameters	*Group 1 (Control)*	*Group 2 (50 mg/kg body weight/day)*	*Group 3 (100 mg/kg body weight/day)*
Sodium (mg/g tissue)	0.75±0.001	0.81±0.001***	0.89±0.001***
Potassium (mg/g tissue)	3.07±0.044	2.92±0.028***	2.56±0.025***
Calcium (mg/g tissue)	0.59±0.008	0.44±0.011***	0.38±0.009***

Values are expressed as mean±S.D. for six rats in each group, ***P<0.001.

Membrane bound enzymes may be inhibited by oxidative stress (20) and degradation products of lipid peroxidation process on the enzyme molecules (21). MeAN induces oxidative stress (22) and membrane lipid peroxidation has been reported earlier (23). The inhibition of Ca^{2+}-ATPase, Mg^{2+}-ATPase activity may be due to the inhibition of oxidative phosphorylation (24). Ca^{2+} in the membrane assist the cross-linking of skeletal proteins. Ca^{2+} binds to anionic sites in the lipid bilayer and alters membrane fluidity (25). Inhibition of $Na^{+}K^{+}$-ATPase activity would be expected to cause a reduction of Ca^{2+} transport out of the cell by means of the Na^{+}/Ca^{2+} exchanger or even Ca^{2+} influx (26) and expected to elevate Na^{+} concentration and reduce K^{+} levels (27). This is consistent with the observations of the present study on the concentration of sodium (Na^{+}), potassium (K^{+}) and calcium (Ca^{2+}) in MeAN ingested rat brain (Table II).

A significant decrease in the activity of NADH dehydrogenase enzyme on MeAN administration was observed (Table I). The decrease in the activity of NADH dehydrogenase enzyme on MeAN administration may be due to reduction in cytochrome oxidase. The reduction in the activities of cytochrome oxidase and NADH dehydrogenase in MeAN administrated rats RBC has been reported earlier (19). MeAN-induced reduction in NADH dehydrogenase activity could be associated with cyanide production by MeAN. Cyanide inhibits the activity of cytochrome oxidase (28). Inhibition of cytochrome oxidase is widely accepted as the mechanism for the lethal effects of cyanide in animals (29).

A significant increase in alkaline phosphatase activity (ALP) in MeAN treated rats was observed when compared to control. Alkaline phosphatase reported to be found mainly in the blood vessels, piaarochonoid and choroids plexus (30). The presence of alkaline phosphatase and anionic charge in the plasma membrane had been known to regulate vascular endothelial transport (31). In extensive brain damage such as in necrotising lesions, severe damage to the vascular endothelium can lead to leakage of this enzyme (32). The increase in alkaline phosphatase may be due to the damage of vascular endothelium during necrosis of brain in MeAN treated rats which is evident in histological studies (data not show).

The present study indicates that the morphological alterations like cell necrosis and changes in cellular homeostasis by MeAN may account for that inhibition of membrane bound enzymes. These results suggest that MeAN besides having toxic side effects also exerts its toxicity on membrane bound enzymes.

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