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The effect of moclobemide, reversible inhibitor of monoamine oxidase-A, on the ethanolized rat brain

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Objective This experiment was carried out to demonstrate the effect of moclobemide on ethanolized rat brain.

Method Thirty male rats, 20-25 g and 20 days old, were used. Rats were fed with a diet (milk) containing ethanol (10%) in ethanol-only treated group and were moclobemide injected (30 mg/kg) in ethanol+moclobemide treated group daily for 21 days.

Results It was found that serum ethanol level in ethanol+moclobemide treated group was significantly higher than in ethanol-only treated group at the end of the experiment. Electron microscopic examination revealed more prominent neurotoxicity in ethanol+moclobemide treated group than in ethanol-only treated group.

Conclusion We concluded that moclobemide decreased the elimination of ethanol. However, more studies are needed to demonstrate its mechanism.

Key words Ethanol, moclobemide, brain, toxicity, electron microscope.

Introduction

Ethanol is a central nervous system depressant. Chronic use of ethanol by humans results in a variety of psychological and physical dysfunctions including a decrease in ability to perform cognitive tasks, loss of ability to form new memories, and cerebral atrophy (1). Chronic ethanol ingestion also results in massive cerebral and cerebellar cortical degeneration in rats (2).

Moclobemide is a new, reversible and selective Monoamine oxidase-A (MAO-A) inhibitor with fewer side effects and has antidepressant properties (3). Moclobemide is safer and more tolerable than other MAO-A inhibitors such as, clorgyline, brotamine, isocarboxaside and harmaline (4). In humans, moclobemide is rapidly absorbed after a single oral administration, and maximum concentration in plasma is reached within an hour. It is markedly bound to plasma proteins. Eighty percent MAO-A inhibition occurs in two hours; the duration of MAO inhibition is usually between eight to ten hours (5).

In this study, we investigated the possible interaction between moclobemide and ethanol. Change in serum ethanol level was measured, and pathological alterations in neural tissues were examined. Brain has been chosen as a histological model to demonstrate the degree of ethanol toxicity.

Material and Method

Thirty male Wistar rats, weighting approximately 20-25 g and 20 days old, were used. Rats were divided into three groups (Control, Ethanol-only treated and Ethanol+Moclobomide treated). Each group consisted of ten rats placed in two cages. Rats were fed ad libitum with milk for a month (Table I). It is suggested that milk is the ideal liquid diet for administering ethanol (6).

Table I. The constituents of the milk used

Substance	Amount
Milk lipids	40.7 g/l
Dextrin-maltose	50.0 g/l
Colin bitartarate	0.15 g/l
Vit B1	0.14 mg/l
Vit B2	1.5 mg/l
Vit B6	0.7 mg/l
Nicotinic acide	0.9 mg/l
Ca pantotenate	3.0 mg/l
Folic acide	0.05 mg/l
Biotine	0.05 mg/l
Vit B12	7.0 mg/l
Vit A	1025.0 IU/l
Vit D	14.0 IU/l
Vit E	1.0 mg/l
Vit K	60.0 mg/l
Ca	1250.0 mg/l
P	920.0 mg/l
Na	500.0 mg/l
K	1500.0 mg/l
M	120.0 mg/l
Mn	10.0 mg/l
Fe	5.0 mg/l
Cu	0.3 mg/l
Zn	4.0 mg/l
I	0.047 mg/l
Cl	1000.0 mg/l
Se	0.03 mg/l
SO4	1000.0 mg/l
Cr	0.01 mg/l
Inositol	20.0 mg/l

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After a month of milk feeding, ethanol (99.9%) was added in milk of ethanol-only and ethanol+moclobemide treated groups for 21 days. Ethanol concentration in milk was 10%. Moclobemide was also injected subcutaneously to the animals in moclobemide+ethanol treated group for 21 days.

Drug used: A solution (0.3%) of moclobemide was prepared in distilled water and injected subcutaneously at the dose of 30 mg/kg every day as used in previous study (7,8).

Analysis of serum ethanol: Serum ethanol level was analyzed by using fluorescence immunoassay method in 3 ml blood taken from the animals at the end of the experiment.

Electron microscopic examination: Brain tissues including frontal cortex and medulla from the right hemispheres of the rats were fixed in 2.5% gluteraldehyde and were postfixed in 1% osmium tetroxide in phosphate buffer. Following dehydration, tissues were embedded in Araldite CY 212. Thin section (500-600 A°) was prepared from araldite blocks on a LKB Nova Ultramicrotome and stained with aqueous uranyl acetate and Reynold's solution and examined and photographed under a Carl Zeiss Em 9S2 electron microscope.

Statistical Analysis: Data were expressed as mean ± SEM. Mann Withney-U test was used for statistical evaluation.

Results

Serum-ethanol level: Mean serum ethanol levels of ethanol-only and ethanol+ moclobemide treated groups are presented in Figure1. Mean ethanol level of ethanol+moclobemide group was significantly higher than those of in ethanol-only treated group ($P<0.001$).

Histological observation: The histological examination of the brain of animals in control group showed normal structure, and no lesion was determined. On the other hand, when the frontal cortex neurons of the ethanol-only treated group were examined, cellular and nuclear membranes were found to be damaged. The cytoplasmic vacuolar degeneration, dilatation of the endoplasmic reticulum cisternae, increased lysosomal dens bodies and lipid droplets, and destruction of the mitochondrial cristae were observed (Fig. 2,3). Some findings were observed in the glial cells. Most importantly, degenerative myelin sheath of medulla were also observed (Fig.4).

In the ethanol+moclobemide treated group, most neurons and glial cells of the frontal cortex had increased cytoplasmic degeneration when compared to ethanol-only treated group (Fig. 5-7). Furthermore, in this group, loss and degeneration in myelin sheath was greater (Fig. 5,7,8). The wide electron lucent spaces and degenerative axonal structures were observed in the neuropil (Fig.5-7).

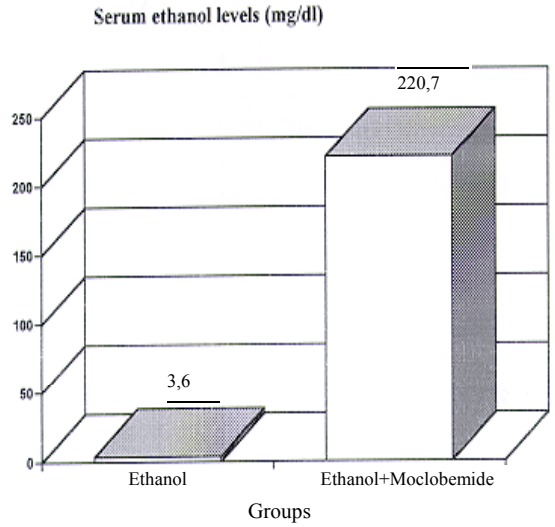

Fig. 1. Histogram of serum ethanol concentrations in ethanol-only and ethanol+moclobemide treated groups.

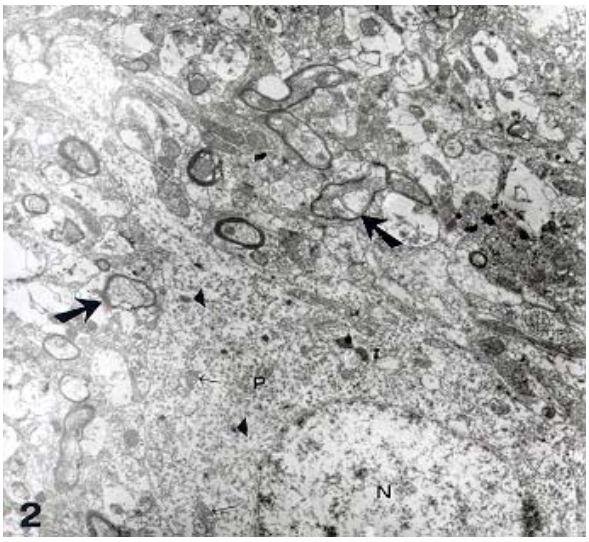

Fig.2. Electron micrograph of a rat brain 21 days after ethanol liquid diet. Perikarion (P), nucleus (N), mitochondria (thin arrows), lysosomal dens bodies (arrowheads), myelin sheats (thick arrows). Uranyl acetate and Reynold's lead stain (X10000).

Discussion

This experiment was undertaken to demonstrate the effect of moclobemide on ethanolized rat brain. It was found that serum ethanol level of moclobemide+ethanol treated group was significantly higher than those of ethanol only-treated group. It was suggested that moclobemide inhibited the elimination of ethanol

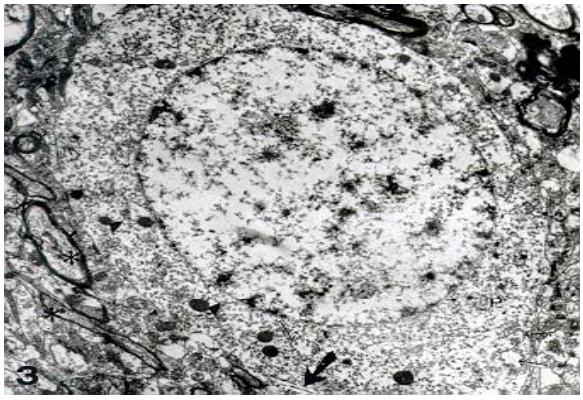

Fig.3. A transmission electron micrograph of the frontal cortex from ethanol-only treated group. The presence of the dilatation of the endoplasmic reticulum (thick arrow), crista disruption in mitochonodria (thin arrows), lysosomal dens bodies (arrowheads) in the cytoplasm of a perikarion (P) are shown. Furthermore, degenerative myelin sheats (asterisks) are present. Uranyl acetate and Reynold's lead stain (X16000).

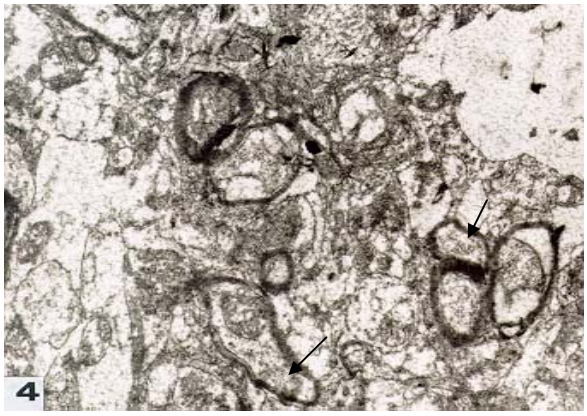

Fig.4. Transmission electron micrograph of medulla from ethanol-only treated group. Myelin degeneration in the axon (arrows). Uranyl acetate and Reynold's lead stain (X10000).

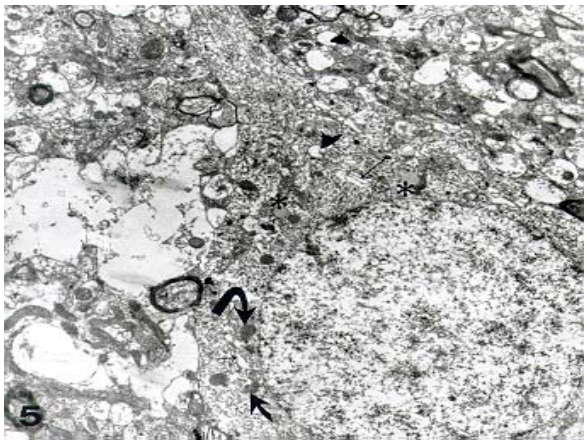

Fig.5. The cytoplasmic degenerations including dilatation of endoplasma reticulum cisternae (thin arrows), crista disruption in mitochondria (arrowheads), and increased lysosomal dens bodies (thick arrows) and lipid droplets (asterisks) are observed in the ethanol+moclobemide treated group. Uranyl acetate and Reynold's lead stain (X15000).

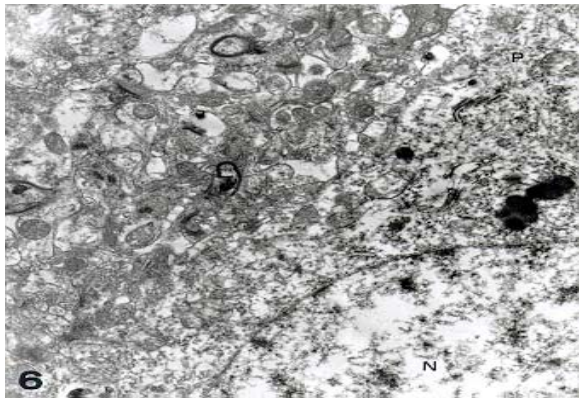

Fig.6. Cellular and nuclear membrane destruction of a neuron from frontal cortex were observed in the ethanol+moclobemide treated group. Perikarion (P), nucleus (N). Uranyl acetate and Reynold's lead stain (X17500).

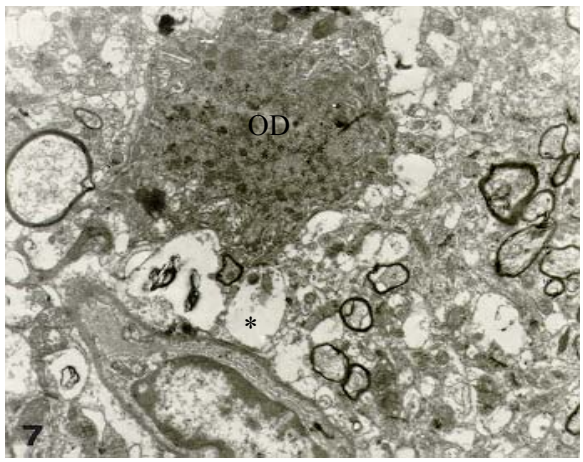

Fig.7. In the ethanol+moclobemide treated group, cellular and nuclear membrane destruction of a dark oligodendrocyte (OD) that shows densely staining cytoplasm, and has more condensed chromation in this nucleus. In addition, edema in the neuropil is seen (asterisks). Uranyl acetate and Reynold's lead stain (X9000).

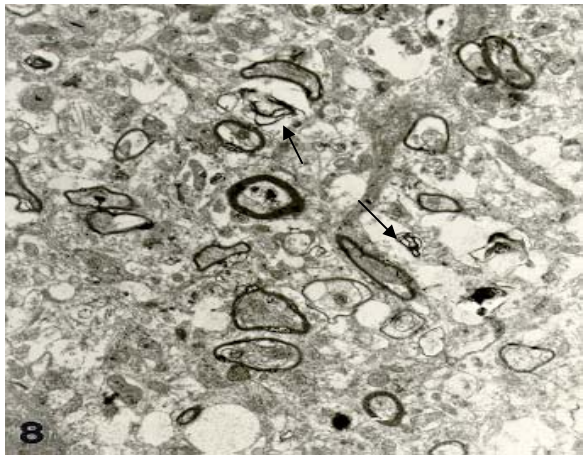

Fig.8. A transmission electron micrograph of a rat brain from the ethanol+moclobemide group treated shows degenerative myelin structures (arrows). Uranyl acetate and Reynold's lead stain (X7500).

from the body (9). Our result consistently showed that somehow moclobemide reduced the elimination of ethanol causing an increase in concentration of ethanol in blood serum. However, mechanism by which moclobemide inhibits ethanol elimination is still unknown. More studies are needed to demonstrate its effect.

It was also found that myelin degeneration and cellular-nuclear membrane disruption were significant in the ethanol+moclobemide group when compared to the ethanol-only treated group. Cortical morphometric studies in the chronic ethanol consumption revealed that neuron loss is more prominent in frontal cortex. Frontal cortex is suggested to be the most ethanol sensitive area in the brain (10). Paula-Barbosa et al demonstrated that the adult rat cerebellum is particularly sensitive to ethanol. Besides the changes in the number and structure of cellular organelles, they observed a progressive degenerative activity including cell death in all crebellar cortical layers, more conspicuous in the granular layer showing a reduction of 30% in the number of granule cells after 18 month of ethanol administration (2). In a study, the effects of ethanol intoxication on the hippocampus was studied in Sprague-Dawley rats; the results confirmed the lethal influence of ethanol on some neurons, and limited ability of the remnant neurons to compensate for neuronal loss (11).

Degeneration of phospholipid-rich myelin structure and biological membranes are closely related to the free fatty acid level that was increased by the free oxygen radicals (12,13). Ethanol abolishes the functions of the antioxidant system leading to an increase in free oxygen radicals; these in turn may result in neuronal death (12-16). Recent experimental studies have demonstrated that oxygen free radicals may be important mediators of brain injury and edema, and pharmacological antagonism of oxygen free radicals shows beneficial therapeutic results (16). Since myelin degeneration and cellular-nuclear membrane disruption were greater in moclobemide+ethanol treated group, this might be due to the moclobemide induced increase in plasma ethanol level that produced free oxygen radicals.

We also found that the cytoplasmic vacuolar degeneration, dilatation of the endoplasmic reticulum-cisterna, increased lysosomal dens bodies and lipid droplets and destruction of mitochondrial cristae were more severe in moclobemide+ethanol treated group than ethanol-only treated group. These results were probably also due to the increased toxic effects of ethanol produced by moclobemide.

It was concluded that moclobemide increases the toxic effect of ethanol by reducing its elimination from the body. This is very important in ethanol addicts. An antidepressant drug usage with ethanol produces more toxic effect on the brain.

References

1. Sheetz, A.J., Marcham, J.A., Fifkova, E. Astrocyte proliferation precedes a decrease in basket cells in mice. Brain Res. 460: 246-252, 1988.
2. Paula-Barbosa M.M., Taveres, M.A.; Cadete-Leite, A., Madeira, M.D., Castedo, J.L., Volk, B. Blood derived phagocytes in the cerebellar cortex of the rat after chronic ethanol consumption. J C submicrosc Cytol Pathol. 21 (3): 585-592, 1989.
3. Berlin, I., Cournot, A., Zimmer, R., Pedarriosse, A.M., Manfredi, R., Molinier, P., Puech, A.J. Evaluation and comparison of the interaction between ethanol and moclobemide or domipramine in health subjects Phycopharmacology Berl. 100 (1):40-45, 1990.
4. Stefanis, C.N., Merz, K.F. Moclobemide (O 11-1163) in longterm treatment. Acta Psychiatr Scand. I 360:67-68, 1990.
5. Nair, N.P., Ahmed, S.K., Kin, N.M. Biochemistry and pharmacology of reversible inhibitors of MAO-A agents: focus on moclobemide, J Psychiatry Neurosci. 18:214-225, 1993.
6. Parale, M.P., Kulkarni, S.K. Studies with alpha 2 adrenaceptor agonists and ethanol abstinence syndrome in rats. Phycopharmacol. 88:237-239, 1986.
7. Da-Prada, M., Burkard, W.P. Hypotensive action and weak potentiation of tyramine effect by moclobemide in rats. Acta psychiatr Scand. 360:106-107, 1990.
8. Schoerlin, P.M., Da-Prada, M. Species-spesific biotransformation of moclobemide: A comparative study in rats and humans. Acta Psychiatr Scand. 360: 108-110, 1990.
9. Stoecke, K., Pfefer, J.P. Absorbtion and disposition of moclobemide in patients with advanced age or reduced liver or kidney function. Acta Psychiatr Scand. 360:94-97, 1990.
10. Harper, C.G., Kril, J.J. Brain atrophy in chronic patients: A quantitative pathological study J Neurol Neurosur Psychiatr. 48:211-217, 1985.
11. Bengoechea, O., Goncalo, L.M. Effects of ethanolization on the rat hippocampus. Neurosci Lett. 123(1):112-114, 1991.
12. Nordmann, R., Ribiere, C., Rovach, H. Ethanol-induced lipid peroxidation and oxidative stress in extrahepatic tissues. Ethanol and Ethanolism. 25:231-237, 1990.
13. Uysal, M., Kutalp, G., Özdemirler, G., Aykaç, G. Ethanol-induced changes in lipid peroxidation and glutathione content in rat brain, Drug Ethanol Dependence. 23:227-230, 1989.
14. Thomson, A.D., Pratt, O.E., Jeyasingham, M., Shaw, G.K. Ethanol and brain damage. Human Toxicol. 7:455-463, 1988.
15. Gverri, C., Grisolia, S. Changes in glutathione in acute and chronic ethanol in toxication. Pharm. Biochem. Behavior. 13:53-61, 1980.
16. Ikeda, Y., Long, D.M. The molecular basis of brain injury and brain edema. The role of oxygen free radicals. Neurosurgery. 27 (1):1-11, 1990.

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