Biolog. Journal of Armenia, 4 (60), 2008

ALCOHOL-INDUCED HYPERHOMOCYSTEINEMIA AND DEVELOPING BRAIN ATROPHY

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In the current study, the effect of maternal alcohol consumption was investtigated on hyperhomocysteinemia and atrophy of the developing hippocampus and cerebellum of offspring. The results revealed that along with significant decrease of brain, cerebellum and hippocampus weights in alcohol receiving animals, the levels of homocysteine in plasma, hippocampus and cerebellum were significantly higher than controls. We observed that pathological raised levels of plasma and brain homocysteine showed significant correlation to brain, hippocampus and cerebellum volume reduction. Raised plasma and brain levels of homocysteine are associated with brain atrophy in alcoholism.

Alcohol - homocysteine - brain atrophy

Ուսումնասիրվել է ալկոհոլի ազդեցությանը ենթարկված առնետներից ծնված սերնդի արյան պլազմայի, հիպոկամպի և ուղեղիկի հոմոցիստեինի քանակությունը և ուղեղի ապաճումը: Ալկոհոլի օգտագործման պայմաններում դիտվել է ուղեղի՝ մասնավորապես հիպոկամպի և ուղեղիկի զանգվածի նվազում, հոմոցիստեինի քանակության ավելացում արյան պլազմայում, հիպոկամպում և ուղեղիկում: Բացահայտվել է համահարաբերականություն հոմոցիստեինի քանակության ավելացման և ուղեղի զանգվածի նվազման միջև, որը վկայում է ալկոհոլիզմի ժամանակ զարգացող ուղեղի ապաճման մասին:

Ալկոհոլ - հոմոցիստեին - ուղեղի ատրոֆիա

В данном исследовании изучалось влияние алкоголя на содержание гомоцистеина в плазме крови, гиппокампе, мозжечке, а также атрофию мозга крыс. Было выявлено, что потребление алкоголя беременными крысами приводило к существенному снижению массы всего мозга, гиппокампа и мозжечка потомства. Наблюдалось значительное по сравнению с контролем повышение уровня гомоцистеина в плазме, гиппокампе и мозжечке. Выявленный нами патологически повышенный уровень гомоцистеина в плазме и мозге достоверно коррелировал с уменьшением массы мозга, что может свидетельствовать об атрофии мозга при алкоголизме.

Алкоголь – гомоцистеин – атрофия мозга

There is evidence that elevated homocysteine levels contributed to neuronal cell injury in various conditions including neurodegenerative disorders (Loscalzo et al, 2002). Furthermore, radiological evidence of temporal atrophy progression in patients with Alzheimer's disease has been found to be significant greater among those patients with higher plasma homocysteine levels (Clarke et al, 1998). Clinical investigations and animal exs haperimentve shown that there is a marked correlation between the occurrence of hyperhomocysteinemia and alcoholism (Marilia et al, 2000). More recent studies have demonstrated that the plasma homocysteine concentration is not only dependent on the type of alcoholic beverage, but especially on the amount of alcohol concentration (Bleich et al, 2000). The degree of the plasma homocysteine levels is strongly determined by the degree of alcoholism (Bleich et al, 2000a). Hyperhomocysteinemia in chronic alcoholism was first reported by Hultberg et al (Hultberg et al, 1993). Who found a significantly higher concentration of plasma homocysteine in a group of alcoholics hospitalized for detoxification than control subjects. Speculatoin whether the increased incidence of stroke found in these patients might be related to the increased plasma homocysteine in that study population. Although the causes for such an increase were not sought in this former study, they hypothesized that such an increase could be related to disturbed folate metabolism. It has been postulated that the observed hyperhomocysteinemia in patients suffering from chronic alcoholism is partly due to their decreased intra-cellular B-vitamin levels, especially folate. It has been known for many years that ethanol has an effect on folate metabolism, which could not be explained by an alcohol-induced low intake of folate. The aetiology of folate deficiency in alcohollism can be ascribed to several causes, such as low dietary intake, poor absorption, decreased hepatic uptake and retention, and increased urinary excretion of folate (Halsted et al, 2002). Folate is a cofactor in one-carbon metabolism, during which it promotes the remethylation of homocysteine-a cystotoxic sulfur-containing amino acid that can induce DNA strand breakage, oxidative stress and apoptosis (Mark and Thomas, 2003).

The present study was designed to investigate the possible link between chronic alcoholic intake by pregnant mother and hyperhomocystein and developing brain atrophy in offspring of rats.

Materials and methods. All procedures on rats were performed according to the "Principles of Laboratory Animal Care" (NIH publication no. 85-23, revised 1985), as well as the specific rules of the "Animal Care and Use Committee", National Medical and Health Service. Adult female wistar rats weighing 220 ± 20 g were maintained under standard laboratory condition, on 12-hr light/dark cycle, with food and water available ad libitom. The rats were then mated with males previously tested as fertile, and were checked for the presence of the vaginal plug the next morning. The presence of a vaginal plug was considered to be indicative of conception, and that hour was designated as day 0 of gestation (GD0). On day GD7 of gestation, pregnant rats were randomly divided into two groups: 1) control group, 2) ethanol group. The control group was only treated with a vehicle (normal sterile saline). Rats assigned to the alcohol group received 4.5 g/kg ethanol (Merck - Germany) solution in saline (20% w/v) subcutaneously once a day. The treatment was started on GD7 and continued through to 21 days postnatal (PN) (lactation period). After 35 days (14 days prenatal and 21 days postnatal) treatment of dams, 8 male pups from each group, were anesthetized with ether.

Pups were weighed, then thoracic cavity was opend and blood samples were directly collected from heart of liters by syringe, mixed with ethylenediamine tetraacetic acid (EDTA) as an anticoagulant and centrifuged at 3000 rpm for 10 min. plasma was separated and stored at -20˚ until analysis.

The pups were killed by decapitation and the brain was immediately removed on ice-cold surface, and weighed.The hippocampus and cerebellum were dissected from the brain and weighed on a digital balance with 0.0001 g sensitivity. Hippocampus and cerebellum from each pup were homogenized in 10mM Tris- HCl (pH 7.4), 5mM EDTA.Two hundred µl of plasma or tissue homogenates was deproteinized with equal volume of 0.4 perchloric acid, and the mixture was centrifuged at 10,000g at 4˚ for 20 min.

The levels of homocysteine in serum and tissues of the subjects were measured by means of high pressure liquid chromatography (HPLC) method using ClinRep® complete Kit (Recipe Chemical and Instruments GmbH, Munich, Germany). Homocysteine was measured as it described by the manufacturer. Flow rate was 1.0 ml/min and column temperature was 30°. Retention times of cystein, internal standard, cysteinylglycine and homocysteine were 2.07, 2.41, 2.76 and 3.29 minutes respectively. Fluorescence detector was set for 385 and 515 nm as excitation and emission wavelengths, respectively. 20 ml of samples or calibrator (ClinCal® and ClinChek®, Recipe Chemical and Instruments GmbH, Munich, Germany) were injected. Calculation of unknown samples was done using the internal standard method via peak areas.

Data analyses

One way analysis of variance (ANOVA) was used to compare the amounts of homocystein levels among groups. In each test, the data were expressed as the mean ± SE and $p<0.05$ was accepted as statistically significant.

Results and discussion. Table 1 shows body, brain, hippocampus, cerebellum weights and ratios of hippocampus weight/brain weight, cerebellum weight/brain weight and brain weight/body weight. As shown in table 1 the body weight in ethanol group show significantly decrease compared control group ($p< 0.05$). Also hippocampus and cerebellum weighs in ethanol group is significant low than control ($p<0.05$).

Table 1. Changes in body, brain, hippocampus and cerebellum weight, brain weight/body weight, hippocampus weight/brain weight and cerebellum weight/brain weight ratios during experiment.

parameters / groups	BW(g)	BrW(g)	HW(g)	CW(g)	BrW/BW (mg/g)	HW/BrW (mg/g)	CW/BrW (mg/g)
Control	41.7±0.31	1.36±0.024	0.077±.0017	0.31±0.0057	33±0.31	55.2±1.2	200±5
Ethanol	34.4±0.69*	1.26±0.02*	0.06±0.0011*	0.25±0.0074*	37±0.8*	43.75±1.1*	170±7.5*

"*" significant at $p<0.05$ with control.Values are presented as the mean ± SE.
BW=body weight, BrW=brain weight, HW=hippocampus weight, CW=cerebellum weight.

In comparison to controls, brain/body weight ratio in ethanol receiving rats was significantly high ($p<0.05$), but ratios of hippocampus weight/ brain weight and cerebellum weight/brain weight is significantly low than control group ($p<0.05$).

The amount of plasma homocystein was significantly high than control grouped. As shown in fig. 1, homocysteine level in cerebellum and hippocampus of ethanol group showed massive increased compare control group ($p<0.05$).

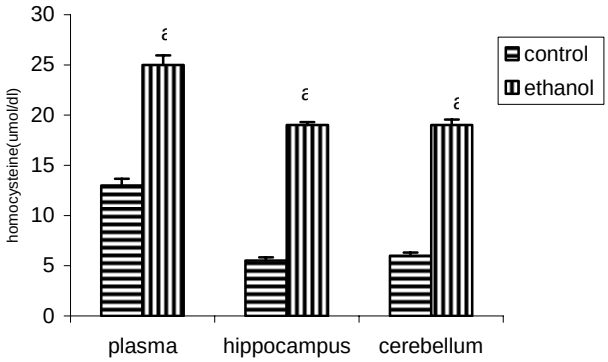

Fig1. Ethanol increase significantly homocysteine concentration in plasma, Hippocampus and cerebellum.
"a" significant at $p<0.05$ with control. The data are presented as group means±SE.

The principle finding of this study is that offspring from alcoholic maternal have 1-greater plasma, hippocampus and cerebellum homocysteine amount's compared non-ethanolic control group, but 2- body, brain, hippocampus and cerebellum weights in ethanolic group were significantly low than control group. There is evidence that chronic alcoholism is associated with hyperhomocysteinemia (Bleich et al, 2000b). The reason for the significant correlation between blood alcohol concentration on the one hand, and plasma homocysteine on the other, regardless of whether beer, wine or spirits had been consumed, are most likely complex ones in alcohol-dependent patients: impairment of remethylation of homocysteine is brought about on account of a dysfunction of methionine synthase, due to an alcohol-induced vitamin deficiency(folic acid, vitamin B12 and B6), as well as a direct inhibition of methionine synthase due to acetaldehyde, the product of oxidative degradation of alcohol (Bleich et al,2000c; Kanh et al, 1987). Hippocampal volume reductions on magnetic resonance imagine (MRI) has been reported in patients suffering from chronic alcoholism (Laakso et al, 2000). Homocysteine is thought to cause CNS damage mainly by way of three plausible but hypothetical mechanisms. One is that homocysteine is directly neurotoxic. This possibility is suggested by the observations that homocysteine is an endogenous compound which is neurotoxic in supraphysiological concentrations. In theory, high brain concentration of either homocysteine or its oxidized derivatives might alter neurotransmission or induce excitotoxicity in neurons, particularly those expressing N–methyl D-aspartate type receptors (Lipton and Rosenberg, 1994; Robert et al, 2004). A second mechanism postulates that homocysteinemia indicates a metabolic disruption in homocysteine and one-carbon metabolism which extends to the brain. An accumulation of homocysteine would increase intracellular S-adenosylhomocysteine (SAH), which is a potent inhibitor of many methylation reactions that are vital for neurological function including the O-methylation of biogenic amines, the methylation of myelin basic protein and the synthesis of phosphatidyl choline. The third possibility is based on the association of homocysteinemia with occlusive vascular disease which may be mediated through damage to the blood vessel wall or impairred blood coagulation. If this was to occur in the brain, then homocysteine-induced cerebrovascular damage could lead to secondary neuronal dysfunction

and degeneration, white matter damage or stroke. In addition certain region of CNS such as the hippocampus and cerebellum, may by particularly to oxidative stress because of their low endogenous levels of vit E, an important biochemical antioxidant, relative to other brain regions(Abel et al, 1995) Moreover, immunohistochemical analyses revealed that Hcy was not distributed uniformly in the brain but accumulated in specific regions, including the cerebellum, the hippocampus and the subventricular zone lining the lateral ventricle (Chung et al, 2003). Such a depressed defense system may be adequate under normal circumstance. However, in pro-oxidative conditions, such as during alcohol exposure, these low antioxidant defenses can predispose the fetal brain to oxidative damage. Interestingly, results of current study demonstrate that hippocampus/brain and cerebellum/brain weight ratios in ethanol group are significantly lower than control group, and reveals that hippocampus and cerebellum were affected more than other parts of the brain.

In conclusion, the data from our study demonstrated a significant positive relationship between mother alcohol consumption in pregnancy and lactation duration and offspring increased plasma and brain homocysteine level, and brain atrophy. However, the mechanisms underlying the neurological damage characteristic of choronic alcoholism are poorly understood.

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Received 13.10.2008