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Investigating the Effects of Maternal Acrylamide Administration on Morphological Changes of Choroid Plexus and Lateral Ventricle in Rat Embryos

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ABSTRACT

Introduction: The potential toxicity of Acrylamide (ACR) in humans had become apparent with the detection of this substance in some processed foods. ACR due to the cooking method are increasingly used.

Aims: This study designed to investigate the effects of maternal ACR consumption on choroid plexus volume and capillaries and ventricular changes in rat embryonic.

Methods: Female pregnant Wistar rats were divided into two groups; an experimental and the control group (n=10). Animals in control group received drinking water while rats in experimental group were orally administered 10 mg/kg ACR solution. Pregnant rats were sacrificed on the 15th day of gestation. Their fetuses were taken out and after head fixation and tissue processing, serial sections were prepared and stained with haematoxylin-eosin (H & E). Choroid plexus and lateral ventricle volume and branches and length of the capillaries were measured and the results were analysed using SPSS version 21 and ANOVA statistical test.

Results: Results showed a significant reduction in Choroid plexus volume and capillaries length in experimental group compared to control group ($p<0.05$), the branches of capillaries showed significant decrease ($p<0.001$). Volume of lateral ventricle showed increase in experimental group compared to control group ($p<0.001$).

Conclusion: Maternal ACR has toxic effect on the nervous system and induces structural changes in the development of choroid plexus and lateral ventricle.

Key words: Acrylamide, Choroid plexus, Capillary, Lateral ventricle

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INTRODUCTION

Acrylamide (ACR), CH_2=CH-$CONH_2$, is a small water soluble with chemical and industrial applications [1,2]. It is used to synthesize polyacrylamide, which is often used in laboratories for gel electrophoresis experiments [3,4]. In daily life, acrylamide can be formed during the cooking and processing of foods [5]. Monomeric ACR is known for its neurotoxic effect and producing nervous system degeneration [6,7]. ACR can undergo oxidative biotransformation by cytochrome P450 [8,9]. ACR also crosses placenta to developing fetus significantly, leading to anomalies [10,11]. The present concern with the neurotoxic property of ACR monomer is related to the numerous usage and increasing production of the chemical for the manufacture of high molecular polymers which enjoy important applications in industry.

Choroid plexuses interface between the blood and cerebrospinal fluid (CSF) and participate in the control of brain homeostasis [12]. Choroid plexus lined by non-nervous epithelium consisting of cuboidal glandular cells, located in the extensive folds [13]. During the development of the CNS the Choroid plexus has an important function in the morphogenesis and stability [14]. Most blood vessels in plexus choroide are wide-calibers (approximately 15 μm) capillaries with thin fenestrated endothelial walls [15]. In the rat, choroid plexuses appear in embryos on day 12. In human, choroid plexuses develop around week 6–7 of gestation [16]. Purpose of the present study was to investigate morphometrical changes of the rat choroid plexus blood vessels and volume of lateral ventricle during development in rats.

MATERIAL AND METHOD

Chemical

ACR (99% pure) and formaldehyde solution was procure from Sigma chemical company.

Experimental design

The Ethics Committee of Shahid Sadoughi University of Medical Sciences approved all study procedures. We used 20 female and 10 male Wistar rats (50-60 days old, 200-220 grams). They were housed 10 per cage and fed standard rodent pellet diet. Drinking water and rodent laboratory food were used to feed. The animal room was kept at 20ºC-25ºC and 50% moisture under 12:12 h light-dark cycle. Female rats were mated with males (2:1) in each cage. In the subsequent day, a positive symptom of mating was confirmed by sperm-positive vaginal smears and the presence of copulatory plugs. The presence of sperm in the vaginal smear was determined at Day zero (D0) of gestation. The pregnant mothers were randomly divided into 2 groups.

Group A consisted of pregnant rats which were given standard diet and drinking water (control group). Group B included pregnant rats which were given 10 mg/kg/day ACR from day 7 of gestation (D7). On the 15^{th} day of gestation, the rats were anesthetized under ether and cardiac perfusion was performed through the left ventricle. In each group, 15 embryos were randomly selected from all pregnant rats and studied. Although the use of acrylamide reduced the average number of embryos, fetal absorption points was not observed in the uterus. The embryos were exited and kept in 10% formaldehyde solution for 10 days.

Tissue preparation and histological analysis

Following routine histological processing, paraffin blocks of head of embryos were collected and sectioned with systematic random sampling. 5 µ-thick samples were obtained, and every tenth section selected from all of head. All samples were stained with hematoxylin and eosine (H & E) method.

Stereological analysis

The whole choroid plexus and lateral ventricle area was viewed on a light microscope at a magnification of 10X with the image projected on a computer monitor. Volume calculations were performed using Cavalieri's principle. A point grid with 100 µ distance between two points was overlaid on the images. Hitting points on the choroid plexus and lateral ventricle were calculated and then the volumes were calculated using the following formula:

$V= t \times a (p) \times \Sigma P/M2$

Where V refers to volume component, t is the section thickness, a (p) is the area of one point (1000 µ), Σ P is the total number of point counted in the component, and M is the linear magnification.

Stereological methods are also used to measure the length of the capillaries following formula:

$L=LV \times V$

Where, L refers to length of the capillary. LV is the length of capillary per unit volume and V is volume of choroid plexus [17,18].

Also the branches of capillaries counted using rectangular eye gratician dimensions 10 × 10.

Statistical analysis

All values expressed as mean ± standard deviation (SD). One way ANOVA were used to evaluate the results. Differences with $p<0.05$ were considered significant.

RESULTS

Sterological studies and morphometric measurements of the choroid plexus showed decrease in volume of choroid plexus and length of capillaries in experimental group compared to control group ($p<0.05$). However, the volume of lateral ventricle in experimental group showed increase in volume compared to control group ($p<0.001$).

There was also a significant decrease in the number of branches of capillaries ($p<0.001$).

These results showed in Figures 1-6.

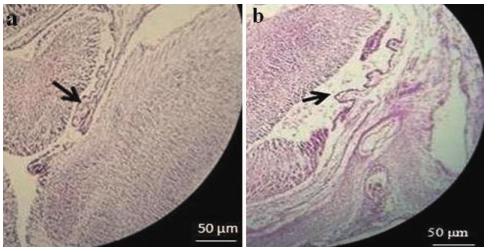

Figure 1: Choroid plexus in 15 day rat embryonic in (a) control group; (b) ACR group (Hematoxyline-Eosin (H & E) staining; Magnification 10X; Arrow showing choroid plexus; Scale Bar 50 µm)

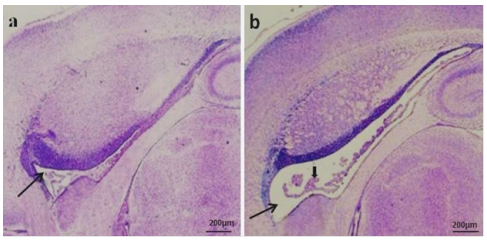

Figure 2: Cross section of lateral ventricle in 15 day rat embryonic in (a) control group; (b) ACR group (Hematoxyline-Eosin (H & E) staining; Magnification 40X; Long arrow showing lateral ventricle; Small arrow showing chorod plexus in ventricle; Scale Bar 200 µm)

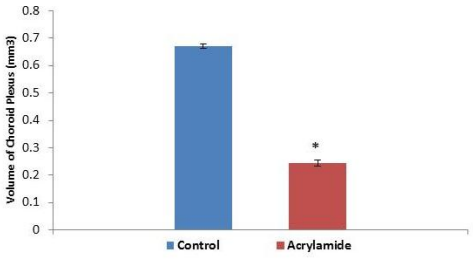

Figure 3: Effect of maternal ACR consumption on volume of choroid plexus (Result showed as mean ± SD; Volume of choroid plexus in ACR group showed significant decrease compared to control group; $p<0.05$)

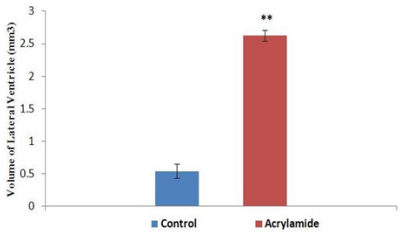

Figure 4: Effect of maternal ACR consumption on volume of lateral ventricle (Result showed as mean ± SD; Volume of lateral ventricle in ACR group showed significant increase compared to control group; p<0.001)

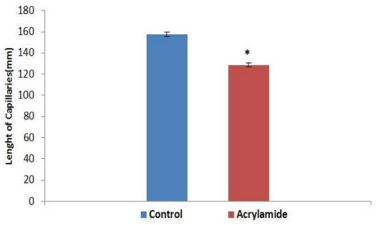

Figure 5: Effect of maternal ACR consumption on length of capillaries (Result showed as mean ± SD; Volume of choroid plexus in ACR group showed significant decrease compared to control group; p<0.05)

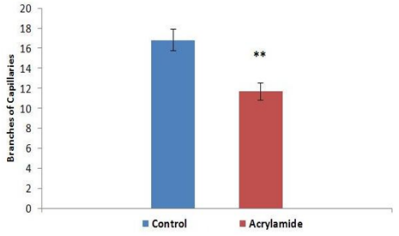

Figure 6: Effect of maternal ACR consumption on count of capillary branches (Result showed as mean ± SD; Volume of choroid plexus in ACR group showed significant decrease compared to control group; p<0.001)

DISCUSSION AND CONCLUSION

Choroid plexus is a sensitive and permeable network in the evolvement and survival of the nervous system which developed at the same time as the organization of the ventricles of brain [19]. Then this plexus specializes and reconcile in being capable to the creation of a blood-brain barrier leads to a continuous fluid secretion spinal cord and ion exchanges between blood and brain [20]. Exposure to ACR leads to prenatal and postnatal malnutrition through its effects on mothers' behaviours [21]. In our study, maternal weight loss in the ACR group was observed in comparison to the control group. It was specified that ACR has neurotoxic effect on neural axons of the peripheral and central nervous systems, however the mechanism of the neurotoxic effect is not discovered yet [22]. In this study, the lower limb weakness was observed in ACR consumption mothers. ACR can pass straight through placenta in experimental animals, reach of fetal tissue and causing developmental disturbance and damage to the tissue [23]. Exposure to ACR disrupts blood-CSF barrier penetrance, which might disrupt protected surveillance along of perturbed immune cell trafficking across the choroid plexus into the CSF [24] among the principal role of the choroid plexus is the CSF generation. However, it is introduced the function of the choroid plexus in the nutrition and conservation of the CNS as the CSF is a chemically constant fluid [25]. The choroid plexus also present a function on the CSF release of medication and substances formed on the brain tissue along of the metabolic reactions, previous studies have shown that evolution of choroid plexus in the mouse begins at about the eleventh day of the embryonic [26]. Research studies have shown that 10% to 15% of ACR in the diet of pregnant women are transmitted to the fetus through the placenta [15]. The results of this study indicate the inhibitory effect of ACR on the development of the choroid plexus and capillaries in the embryo of mothers consuming this substance. It can be concluded that the use of ACR and exposure to it can lead to changes proper functioning of the choroid plexus during infancy and adulthood.

According to previous studies ACR induces premature senescence in endothelial cells [27]. In this study we demonstrate reduction of capillary branches of choroid plexus and reduction in the number of branching. This may be due to cell death in vascular endothelial cells, tissue damage, and disturbing the balance in tissue proliferation.

Ventricular enlargement causes elongation of tissues around the ventricles, which will eventually lead to abnormalities in the fetus and infants. Lack of immigration and evolution cells of the nerve tissue can cause obstruction and extra fluid in nervous system. Ventricles are gathered, which is one of the triggers as Hydrocephalus.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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