Original Article

Effect of Oral Morphine Consumption in Female Rats on Development of Brain Cavities, Central Canal and Choroid Plexus of Their Embryos

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Abstract

Objective: Previous studies have shown that morphine consumption during pregnancy may delay embryo development or cause abnormal nervous system function. The present study focused on the effects of maternal morphine consumption on brain cavities and central canal development in Wistar rats.

Materials and Methods: In this study Wistar rats (average weight: 170-200 g) were used. The experimental group, after pregnancy, received 0.05 mg/ml of morphine by tap water while the control group received water. On the 17th day of pregnancy, the pregnant animals were anesthetized by chloroform and embryos were surgically removed. The samples were fixed in 10% formalin for four weeks. Then, tissues were processed and sectioned. Sections were stained with hematoxylin and eosin (H&E) and examined for ventricle, central canal and choroid plexus development by light microscopy and MOTIC software.

Results: Severe reductions of the third and lateral ventricles were observed in the experimental group. In addition, an increase in the choroid plexus (CP) area in the experimental group with regards to the control group was identified.

Conclusion: The study showed that oral morphine consumption lead to reduction in the third and lateral brain cavities and an increase in the CP area. This defect may cause behavioral changes observed in the F1 generation from addicted pregnant animals.

Keywords: Brain, Cavity, Choroid Plexus, Morphine, Wistar Rats

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Introduction

Affinity and addiction to drugs has spread worldwide and the side effects of addiction not only affect the drug consumer but also have an indirect impact on others, in particular addicted mothers' embryos. Many behavioral problems among addictive mothers' infants have shown the effects of opioides have on developing embryos (1, 2). It is necessary to study the function of drugs in animal trials, particularly in embryos.

Since the central nervous system is the body organizer center, thus a disorder in its cells cause malfunctions in other body systems. Previous studies have shown that oral morphine administration has destructive effects in the evolution of the neural tubes of laboratory rats and in the evolution of the cerebellum in small laboratory mice (3-5). In this research, the effect of oral morphine administration on brain cavities and the central canal were studied in rats. Since the brain cavities and central canal play an important role in cerebrospinal fluid (CSF) transition and neural cell development (6, 7), therefore a disorder in CSF natural transition in brain cavities and the central canal create an interference in the development of neural cells. Therefore it is necessary to study the effects of opioid materials on brain cavities and the central canal in the embryos of addictive mothers. The choroid plexus (CP) plays a key role as the most important provider of nutrients for the development and functioning of neural systems (1). The main function of the CP is CSF production and secretion of this fluid into brain sinuses by ependyma cells. About 10% to 30% of the CSF is of a non-CP origin (8, 9). CSF synthesis is a bilateral process. The CSF is

synthesized and secreted by the CP, then it flows through the lateral ventricles and finally, passes from the fourth ventricle, where some of the CSF reabsorbs into the subarachnoid space and the remainder flows to the central canal. Probably, morphine cause to disorder in secretory function of ependymal cells (6, 9, 11).

Johnson et al. in several experiments on sheep, have proven that the cavernous sinus vein is the place for absorption of the CSF and these veins are located as a pair on both sides of the pineal gland on the basin sphenoid bone. Absorption or secretion of CSF by CP causes hydrocephalic malformation. Due to this malformation the surface of brain cavities increases and thickness of brain tissue decreases (12 , 13). Microscopic examinations have revealed that the structure of CP has evolved greatly in mammals, the surface area of the CP is increased by numerous villi, each villus consisting of a single continuous layer of cuboidal epithelial cells overlying an extracellular stroma surrounding the vascular central core. Choroidal epithelial cells are derived from the ependymal lining of the ventricles and the blood vessels derives from a vascular fold of the piamater termed _tela choroidea.The CP is highly vascularized leading to a good blood supply, that e.g. in rats is almost 10 times greater than the flow to the cerebral cortex (14, 15). Previous research has shown that oral morphine administration has a disruptive effect on the development of different parts of the embryo and nervous system (3-5). According to the important role of the CP in controlling the secretion and absorption of CSF in addition to neural system function which has a direct relationship with the development and survival of the embryo, therefore the present research examined the effect of oral morphine administration on the CP, brain cavities and central canal development in Wistar rat embryos.

Materials and Methods

Wistar rats, with an average weight of 170-200 g, were used in this study. Two rats were housed per cage at a temperature of 24 ± 1ºC with natural light periods (12 hours light/dark). Sufficient food and water were available for rats during the experiment. Rats were maintained in an animal house at Baqyiatallah Medical University. Animal experiments carried out ethically.

In this study, oral morphine sulfate (Tamad Co., Iran) was used. The rats were divided into two groups of six rats per group. A total of 12 female rats in dual groups copulated with adult male rats. After pregnancy confirmation (observation of a vaginal plug and the existence of sperm in the vagina), they were separated from male rats the following morning and kept in the same dual-groups. Thereafter (day 0 of pregnancy), the experimental group received a daily dose of 0.05 mg/ml (5 mg morphine in 1000 ml potable water from the city pipeline for six rats). The amount of consumed morphine in 10 ml water for every 100 g of the rat's weight was computed however attempts were made to make available the amount which the animals needed. On the 17th day of pregnancy, rats were anesthetized by chloroform. Embryos and uteruses were separated from the mother rats and transmitted to a 10% formalin solution for one week and changed the solution. After this phase, embryos were separated from the uterus' endometrium, put in the tissue processing machine and prepared for molding. For molding, the embryos' heads were separated from the trunks and placed in paraffin. Then, the blocks were serially sectioned by microtome (FIST, Germany) into frontal and transverse sections, with thicknesses of 5µm. Sections were put on slides and dyed according to the hematoxylin and eosin (H&E) method (16). After dying and preparation, slides were examined by light microscope. The areas of the ventricles of the central channel and CP in the experimental and control groups were measured by MOTIC software (4, 5). The machine employed consisted of a microscope connected to a computer and a monitor by software. This software is capable of various measurements, in addition to providing the possibility for photography of slides. The cells in each layer were counted and their numbers in the control and experimental groups were compared to each other.

Statistical analysis

Results were reported as mean ± SEM. Differences between group means were calculated by a one-way analysis of variance (ANOVA) and post-hoc Duncan test using the SPSS/PC computer program (version 11.0). Statistical significance between the two measurements was determined by the two-tailed unpaired sample t test. Results were considered statistically significant at $p<0.05$.

The brain cavity areas, central canal and number of CP were compared in the experimental and control groups. Tissues were measured by Motic soft-ware.

Results

Data showed that the effect of oral morphine administration in addictive pregnant rats caused an increase in the CP surface and ependyma cells (Figs 1, 2) and a significant decrease on the lateral cavities and third ventricle surface (Fig 3) in 17 day old embryos in the experimental group when compared with the control.

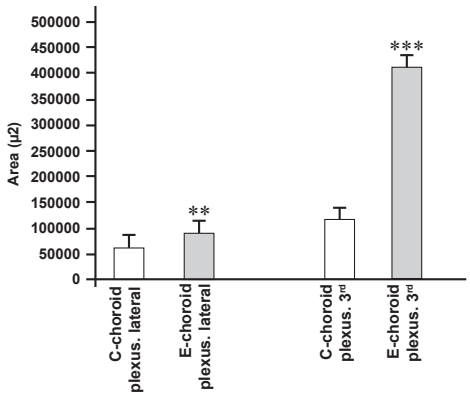

*Fig 1: The effect of oral morphine consumption in the lateral and third CP area in 17 day old embryos. Data are stated as mean ± SEM. The numbers of samples in each group were six. ***P<0.001 & **p<0.01 are the indexes of significance of the third and lateral CP in the experimental (E) group in comparison with the control (C) group.*

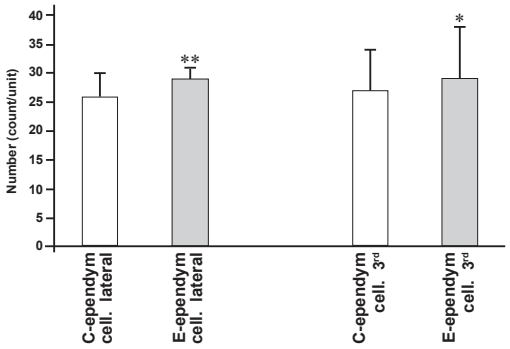

*Fig 2: The effect of oral morphine consumption on the number of ependyma cells in the lateral and third CP brain cavity in 17 day old rat embryos. Data are stated as mean ± SEM. The numbers of samples in each group were six. P<0.05 and **p<0.01 are the indexes of significance for the number of ependyma cells in the third ventricle and lateral CP in the experimental (E) group in comparison with the control (C) group.*

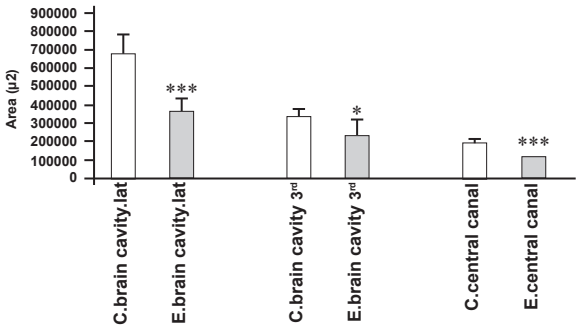

*Fig 3: The effect of oral morphine consumption on the lateral cavities area, third brain cavity and central canal (ependyma canal) in 17 day old embryos from rats. Data are stated as mean ± SEM. For each group, six samples were used. ***P<0.001 and *p<0.05 are the indexes of significance for the third and lateral cavities, and ependyma canal in the experimental (E) group in comparison with the control (C) group.*

Quantitative observations.

Measurements have shown that embryos from the experimental group mothers had less ventricle and central canal surfaces, but their CP surfaces showed a slight increase in comparison with that of the control group (Fig 4-7).

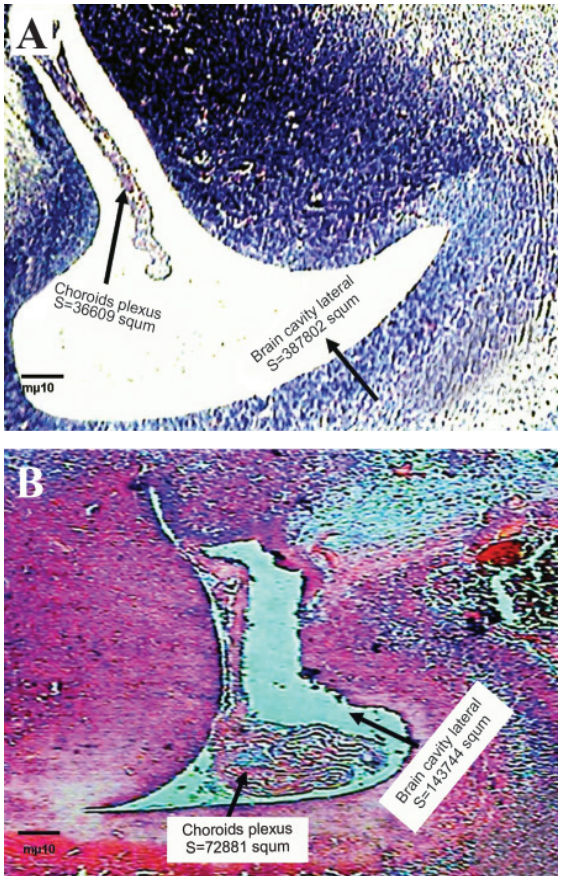

Fig 4: Lateral cavities along with CP in the control group (A) and experimental group (B). Frontal section (×40). Significant differences were shown between two images in the areas of the brain cavity and the extent of CP in the experimental group. S=surface.

Discussion

The results of this study showed the restrictive effect of morphine on brain cavities, central canal development and CP. Morphometric measurements showed that embryos of the experimental group mothers have less lateral cavity, third and central channel surfaces while the lateral cavity of their CP surface and the surface of third cavity increased in comparison with the control group (Figs 1, 3). We performed tissue examination according to two methods to decrease experimental error and to endorse the accuracy of our findings. The morphometric measurements were examined with both transverse and frontal sectioning.

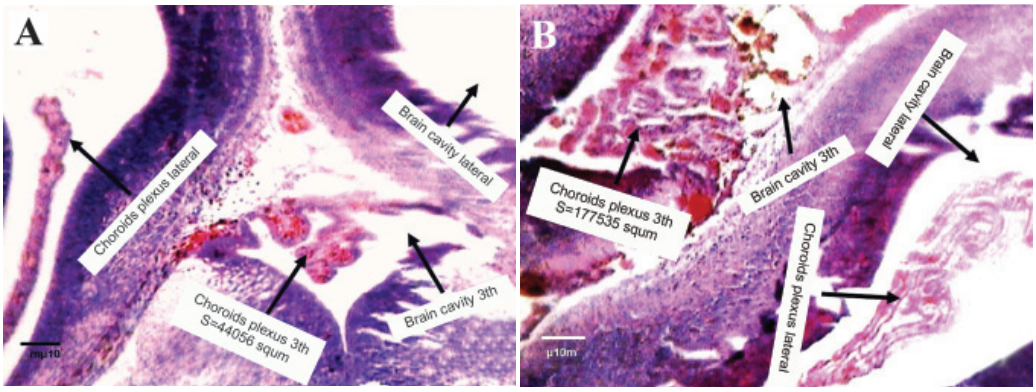

Fig 5: Lateral and third cavities along with CP in control group embryos (A) and experimental group embryos (B). Frontal section (× 100). Significant differences were shown between the two experimental and control groups with regard to cavity brain areas and extent of CP.

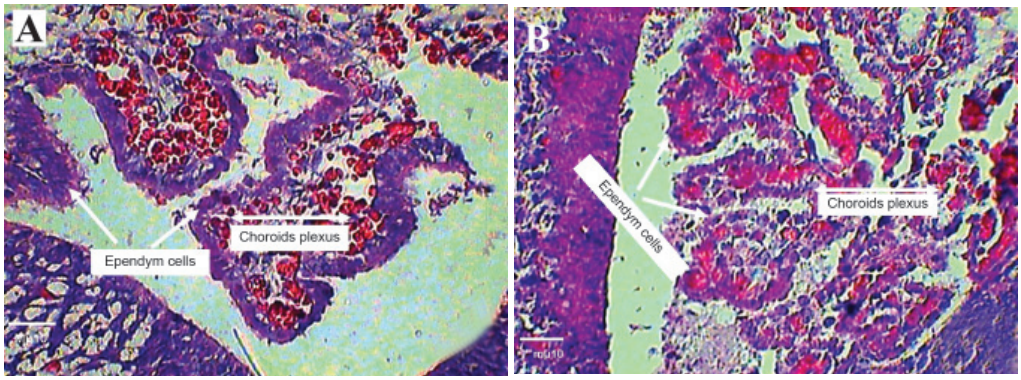

Fig 6: CP ependyma cells in the control (A) and experimental group embryos (B). Transverse section (× 400). Significant differences were shown in the extent of CP surface of the third cavity between the experimental and control groups.

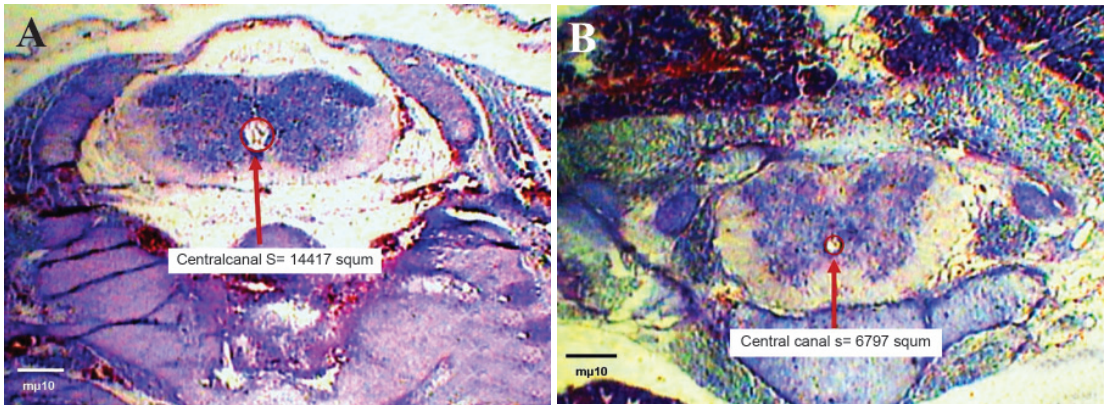

Fig 7: Central canal in the control (A) and experimental group embryos (B). Transverse section (× 100). The size of the central canal surface of the two groups can be seen in the figure. Significant differences were shown in tissue solidarity and the size of the central canal surface in the two groups.

The results of the examinations in both sections showed a decrease in the brain cavity surfaces, increase in CP surface and decrease in CP tissue solidarity in the experimental group in comparison with the control group. Additionally, CP plays a main role in the nourishment and relief of brain cells (9, 17) and its main function is the absorption and secretion of CSF into and from brain cavities (12,18). Decreament and increament in the synthesis of CSF by CP is the cause of many malformations. For example, hydrocephaly is related to an increase in secretion and synthesis of CSF by

CP and finally an increase in the surfaces of the brain cavities surfaces (11, 13). Because of the small molecular size and high morphine lipophility, it passes easily through the placental barrier reaching the embryo. Opioid receptors on the placental villi and vessels have been identified (19-21); receptors stimulated by morphine can result in vessel contraction and a decrease in blood supply to the embryo (21-23). Previous examinations have shown that oral morphine administration during pregnancy can delay placental development (13, 23), in particular its embryonic part which has more villi, blood vessels and opioid receptors (17, 18). A recent study has shown that oral morphine administration in CP increases the ependyma cell surface of CP and increases blood in the CP vessels (Figs 1, 2). The effect of morphine on opioid receptors which exist in the blood vessel endothelium of the CP result in proliferation of ependyma cells, blood vessel contraction and causes disorders in the secretion of CSF by ependyma cells which lead to excessive bleeding and an increase in the CP surface (24-26). Disorders in CP secretion reduce CSF, ultimately causing a decrease in the lateral and third cavities and central canal (Fig 3).

The side effect of this malfunction on the deficiency in supplying oxygen and decrease in brain cell nourishment are considered the main factors which delay growth of the neural system (22, 23). Colin and Doppler showed, morphine was resulted in placental villi and mesencephalic vessles contraction. Thus morphine administration causes the secretion of stress hormones such as corticosteron (11, 19, 22). Which lead to an increase in the concentration of blood plasma corticosteron (27). The activity of corticosteron in the presence of morphine causes an increase in blood pressure and excessive bleeding in the CP in rats. Corticosteron is effective in the unnatural propagation of less differentiated cells by shortening the interphase stage, therefore not allowing the cell to have enough time to synthesis proteins, replicate chromosomes and insufficient growth which causes unnatural proliferation and disorders in natural cellular function. Results have shown (28, 29) that when male and female rats chronically receive morphine, increases in corticosteron activities are seen (23, 30). Probably the increase in ependyma cells is in relation with an increment of the corticosteron concentration due to morphine administration (29, 31, 32). The decrease in CSF can be in relation to morphine function and increase in corticosteron concentration. In the present study, reduction in CSF flow has caused a decrease in the surfaces of the brain cavities and central canal. Since they play a key role in neural cells for the transfer of nutrients, thus disorders in the brain canal and cavities can delay neural cell development (9, 13, 17).

In the present study we observed severe morphologic and morphometric disorders such as decreases in the central canal surfaces. This disorder in the brain cavities and central canal confirmed the effect of morphine as a disorder in the natural function of CP, including the synthesis and secretion of CSF. The main function of ependyma cells of the CP in the four brain cavities is the steady production and secretion of CSF such that it flows into the brain cavities and provides nutrients for neural cells. The decrease in CSF and its relation with a decrease of brain surfaces has prompted us to investigate the relation between the central channel and decrease in CSF. In this study, the decrease of the central canal surface in the experimental group was observed. The CP surface is vast and vascular but because of a disorder in the secretory function of CP and decrease in CSF flow, the surface of the central canal in the experimental group when compared with control group decreased.

One general result from this experiment is that the oral morphine effect causes a deficiency in brain cavities and CP genesis in 17 day old embryos of the Wistar rat in the experimental group in comparison with the control group. This deficiency is observed as the decrease in the surfaces of the lateral and third cavities, in addition to increases in the surface of related CP. On the other hand, the addiction effect can cause deficient neural system development in embryos of pregnant human mothers.

Conclusion

Morphine causes abnormal proliferation of ependyma cells following disturbances in ependyma cell function, increase in CSF secretion, and absorption and decrease in brain cavities and the central canal surface. According to studies, morphine administration can cause lateral and third cavity defects as well as defects in the central canal and CP in addictive Wistar rat embryos. This result may also be true for humans although behavioral disorders in infants or embryonic abortion from addictive pregnant mothers need to be further studied.1,13 The results of this malfunction in 17 day old addicted embryos caused delays in cavity and central canal development, and finally defects in neural system development.

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flict of interest in this article.

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