

Study of Vincristine Effect in the Gestation Period on Mice Cerebellum Formation

¹Eilyad Issabeagloo, ²sajjad hejazi, ³mohammad taghizadieh

¹*Department of Pharmacology, Medical Sciences Faculty, Tabriz branch, Islamic Azad University, Tabriz, iran.*

²*Department of Basic Sciences, Faculty of Veterinary medicine, Tabriz branch, Islamic Azad University, Tabriz, iran..*

³*Department of pathology, Medical Sciences Faculty, Tabriz branch, Islamic Azad University, Tabriz, iran.*

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ABSTRACT

Vincristine is alkaloid that was derived by vinca-rosea and it is administered for inhibition of division of malignant tumor cells with high proliferation. Occurring of malformation in embryos was proved in pregnant mothers who received this drug (23-85 percent). However, there was no adequate information about its toxic effect in newborns cerebellum structures. Will considering to B.P. Barrier passing and cytotoxic effect, rate of destructive effects to formation of cerebellum in newborns was demonstrated in this study 20 female Mice were pregnant with male Mice and served as two groups (control and experimental) accidentally. The Mice of experimental group received this drug 3mg/kg in days 10 and 15 pregnancy (IP). In end of pregnancy duration 48 newborns were selected for sampling from control and grooming accidentally and after performing of histotechnique and staining H&E were considered under light microscope. It was used from T-test and SPSS software for analyzing data obtaining from quantative parameters. In base of morphologic observations performing in grooming group, it was obtained significant decrease in weight, skull size and newborn growth in comparison with control group ($P < 0.001$). In base of microscopic observations from primary growth of cerebellum, experimental group in comparison with control group, it was no tissue regulation in molecular, porkinje and granular layers and it was in primary formation. White matter of cerebellum was seen with increasing in interstitial space and decreasing in compaction of neuroglia cells accompany with deficiency in dismyelination of nervous fibers. Occurring of apoptosis were seen in epithelial cells of choroid network and white matter neuroglia cells in experimental group, in base of obtaining conclusions we can conclude that effects of anti-mitosis drugs con include inhibitive activity of drug to difference and proliferation of cortical cells of cerebellum and its formation ultimately and it causes to support of apoptosis induction in choroid network cells and cerebellum.

Key words: Cerebellum, Mice, Vincristine

Introduction

Vincristine is one of vinca alkaloid that is derived from madagascar perivinkle plant (Vinca-rosea) that binds to tubulin and inhibit its polymerization into microtubules, preventing spindle formation in dividing cells and causing arrest at

metaphase [19,3]. It inhibits cells mitosis cycle so that it considered as one of the malignant cells mitosis inhibitor [2,18]. Cerebellum from terms of embryonic formation and genesis has metencephalon origin. Cerebral neurons shape and their spatial arrangement determined during development of central nerve system was the same in all vertebrates

Corresponding Author

Eilyad Issabeagloo, Department of Pharmacology, Medical Sciences Faculty, Tabriz branch, Islamic Azad University, Tabriz, iran.

E-mail: e.issabeagloo@gmail.com; Tell: +989144079927

and abnormality of this area caused motor disorders [13]. In previous studies has been expressed that cerebral emergence in prior was as a mass that is detectable on days 14 to 17 of gestation and cerebellum primary shape is detectable from day 17 of gestation as (foliated shape) [4]. In a study on rat cerebellar development has been mentioned that X-ray and cytotoxic drugs have destructive effect on regulating cell development, migration and differentiation of cerebellar 3-ply cortex [11]. In another study on neuroepithelial cells migration in mice subsequent poisoning with vincristine during pregnancy has been expressed that this drug can cause abnormalities in the cephalic fold and optic chiasma which is seen from blight to Asymmetric growth [17]. Purkinje cell damage by many post puberty diseases such as seizure, Alzheimer's and Huntington considered relevant that called Cerebellar congenital affective syndrome. This syndrome is associated with visual and dialect failures [15,16]. Studies have shown that 23 - 85 percent of pregnant mothers who have received this drug, occurrence of malformation are seen, however, there is no sufficient information regarding the drug teratology effects on fetus [4]. In this study have been tried to show the known cytotoxic effects of this drug on cerebellar formation by using this drug ability to passing across of blood-placenta barrier.

Material and method

In this study Swiss male and female mice weighted 30±5g were provided. Females during proestrus cycle were kept near males. Vaginal smear assessment was done at 8 AM so that with observing sperm or formed vaginal plaque in smear was considered day one of gestation. In sum 20 mice were gestated by in turn and in continuo gestated mice were allocated by chance in two control (n=10) and treatment (n=10) groups. Considering the severity of the teratology occurrence followed by drug use during organogenic period [6,14] and onset of cerebellar growth in that period, vincristine was administrated intraperitoneally on days tenth and fifteenth of pregnancy [10,12] at the dose of 3 mg/kg for the treatment group [1,2,5]. The control group in those days was treated by the injection of normal saline. In the end of the study, totally 48 neonates were selected by chance from both control and treatment groups. Samples after completion of histotechnique stages are stained as hematoxylin and eosin and were studied with light microscope. In this study for comparison of data obtained used of Mean±SEM and T-test to analysis data.

Results and discussion

Morphology Observations on neonates of

treatment group revealed significant reduction in weight, skull size and growth of the neonate compared with controls, respectively P<0.001 table 1.

Table 1: data obtained from external morphological observations after twice intraperitoneal administration of vincristine on days 10 and 15 of pregnancy. Data were presented as Mean±SEM. (N=20)

Parameter	Control group	Treatment group
Neonate weight (g)	1.63±0.04	0.97 [*] ±0.04
Neonate length (mm)	24.76±0.51	19.97 [*] ±0.65
Skull width (mm)	7.77±0.13	2.79 [*] ±0.11
Skull length (mm)	10.56±26	9.13 [*] ±0.18

*Asterisk in superscript indicates significant difference with control group (P<0.001).

Based on microscopic observations of early growth in the control group neonatal's cerebellum, three cell layers of cerebellar cortex visible and were separated from each other. Cerebellum was its primary foliated form and expansion was seen in pia mater surrounding the cerebellum. In treatment group, molecular, purkinje and granular cell layers were not histology regularity than control group that cerebellar so no foliated shape and was seen to its primary form (Fig 1,2).

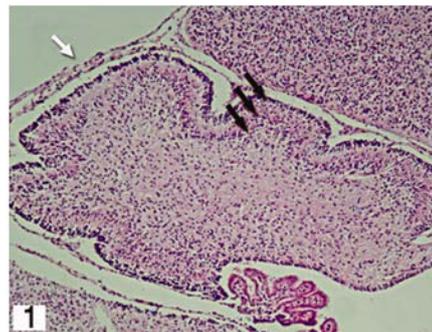


Fig. 1: microscopic view of cerebellum tissue with three cortical layers (black arrow) and meninge (white arrow) of control group. (H&E ×10).

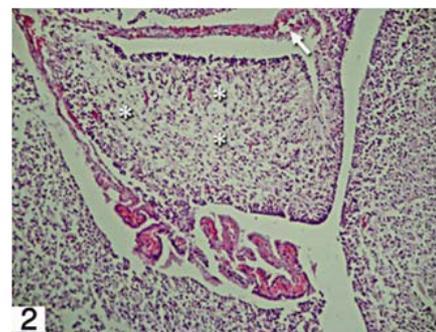


Fig. 2: microscopic view of cerebellum tissue, meninge (white arrow) and interstitial space of cerebellum white matter (asterisk) of treatment group. (H&E ×10).

Presence of Purkinje cells associated with cerebellum cortex in treatment group is rarely seen compared with condense purkinje cells in control group (Fig. 3,4). Survey of cerebellum white matter in the treatment group compared with normal cerebellum white matter tissue in control group demonstrated defects in the neuropil tissue which was accompanied by decreased staining and increased interstitial space and decreased neuroglia cell condensation. Also failure in myelination of nerve fibers in cerebellum white matter in treatment groups was observed (fig 3,4). In study on Meninges surrounding the cerebellum in the treatment group compared with the normal state of control group meningeal tissue, pia matter was observed as hyperemia and edematous (fig 3,4).

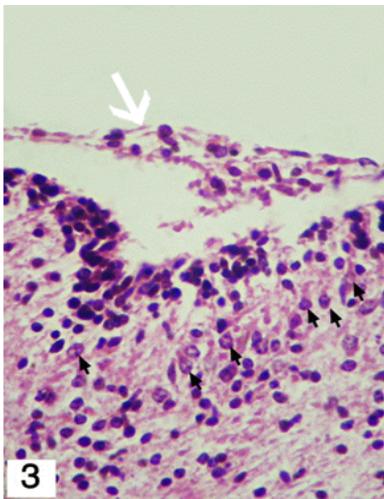


Fig. 3: microscopic view of cerebellum tissue with purkinje cells condensation (dark arrow) and meninge (white arrow) of treatment group. (H&E ×40)

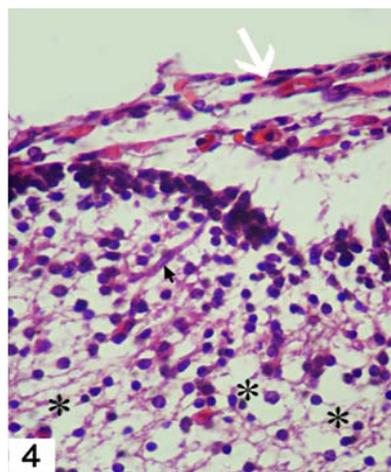


Fig. 4: microscopic view of cerebellum tissue with purkinje cells (dark arrow), meninges (white arrow) and interstitial space of cerebellum white matter (asterisk) of treatment group. (H&E ×40)

In observations of the fourth ventricle structure surrounding the cerebellum in control group, choroid plexus was fully developed and choroid plexus epithelial cells was normal status. In treatment group, structure of the fourth ventricle choroid plexus was observed hyperemia and choroid plexus epithelial cells due to necrotic pretense, lost his integrity and choroid plexus tissue was observed separate. Necrotic debris with fibrin infiltration subsequent choroid plexus injury in forth ventricle interstitial space obviously seen [6,7].

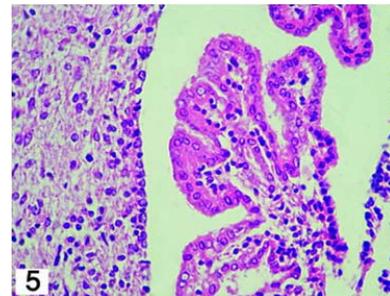


Fig. 5: microscopic view of choroid plexus of forth ventricle in near the cerebellum tissue in control group. (H&E ×40).

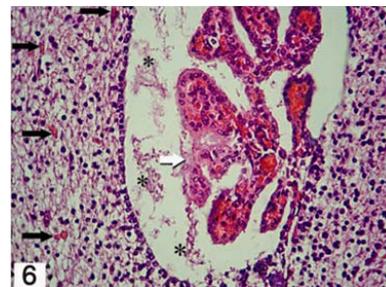


Fig. 6: microscopic view of choroid plexus of forth ventricle in near the cerebellum tissue with hyperemia and necrotic expressions (white arrow), fibrin infiltration and necrotic debris in ventricular space (asterisk) and expanding of vessels in cerebellum tissue (black arrow) in control group. (H&E ×40).

Occurrence of apoptosis in the choroid plexus epithelial cells was from other observations in the treatment group compared with the control group. Apoptosis with hypereosinophilic cytoplasm, condensation and fragmentation of nuclear chromatin and finally formation of apoptotic bodies was visible (fig 7). The occurrence of apoptosis in neuroglial cells of cerebellum white matter was seen scattered basis (fig 8). All changes indicated apoptosis in neurons of cerebellum cortical layer were rarely visible.

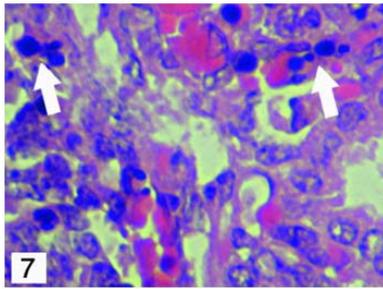


Fig. 7: microscopic view of epithelial cells of fourth ventricle choroid plexus, chromatin segmentation and formation of apoptotic bodies of epithelial cells (white arrow) of treatment group. (H&E $\times 160$).

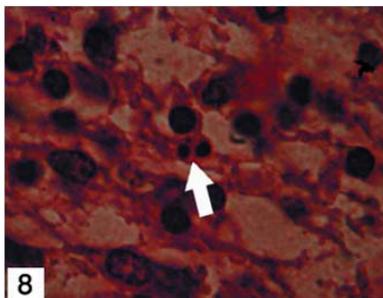


Fig 8: microscopic view of epithelial cells of fourth ventricle choroid plexus, chromatin segmentation and formation of apoptotic bodies in glial cells of cerebellum white matter (white arrow) of treatment group. (H&E $\times 160$).

Discussion:

Most injuries on cerebellar structures during pregnancy in experimental models, fetal inflammation, brain ischemia and prenatal studies, are examined. In this study also based on observations, intervention descriptions revealed to us that the structure of the cerebellum from organogenesis to late gestation period significantly is prone to irreversible damages. In a study conducted by Haton *et al.*, [7] on development of sheep fetus cerebellum with intervention of endotoxins had been done indicated that damages during late pregnancy period is irreversible [7].

In a study by neky and shreni, [11] on occurrence of malformation in the process of developing cerebellar tissue, has been expressed that the intervention of antimetabolic activity drugs or X-ray in the neonatal rats will make the first damages in the pia mater of meninge [11]. In current study based on done observations, the damages resulted from vincristine on treatment group neonates expressed as hyperemia and oedema in pia matter. Studies by Kamper and Bowman [8] and Kern [9] in neuropathological field has been mentioned that a

high percentage of purkinje structure injuries is related to reduction in number and sometimes the size of purkinje cells [8,9]. In this study the status of purkinje cell condensation in treatment group compared with the control group was expressed that resulted from the deleterious effects of vincristine on mitotic and cellular activity of purkinje cells. Prakesh *et al.*, [14] in a study on the tenth and twelfth days of pregnancy (organogenic period) on mice have shown that cytotoxic drug Cyclophosphamide had a significant inhibitory effect on choroid plexus epithelial cells and induces apoptosis in the mouse brain cells [14]. In this study also the vincristine effect on epithelial cells of choroid plexus in treatment group, with nuclear chromatin fragmentation (apoptosis) were showed and also expressed the effect of this drug in cerebellar white matter cells with the occurrence of apoptosis.

Conclusion:

Based on the obtained results and previous studies can be concluded that such effects of antimetabolic drugs (cytotoxic) could include:

- 1- This drug has inhibitory activity on cell proliferation and differentiation of cerebellar cortical layers.
- 2- Be confirmatory activity on apoptosis induction in cells of choroid plexus and cerebellar tissue.

Overall this study, new events to prove the mechanism of induction of backwardness in the cerebellum formation of mice neonates' who are affected by chemotherapy (vincristine) during intrauterine life was presented and also apoptosis induction and disorganization of cell structure of the cerebellar tissue also planned.

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