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Prenatal treatment with metronidazole induces cerebellar folia alteration in guinea pig fetuses

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Abstract: The most sensitive period in brain development is during prenatal life. The use of antibiotics in pregnancy is still controversial. Recent studies revealed the high neurotoxic potential of the antibiotic and antiprotozoal medication, metronidazole. However, there are insufficient data from animal studies about prenatal treatment effects. We investigated the effect of prenatal treatment with metronidazole on cerebellar development in guinea pigs. Treatment with metronidazole was performed from the 42nd to the 49th day of gestation. On the 50th day of pregnancy, all dams were killed, and the cerebella of the fetuses were analyzed. Gross cerebellar changes characterized by malposition of the folia with partial atrophy were found in 12 of 19 fetuses in the experimental group, but in none of 20 control fetuses that received saline. The most affected were folia VII with depletion of the areal fraction of the external granular layer, molecular layer and the internal granular layer. Purkinje cells displayed cell distortion with loss of normal dendritic polarity. The investigation revealed cell depletion, with a disturbance of the cytoarchitectonic of the cerebellar cortex and folia alteration.

Keywords: metronidazole; neurotoxicity; neuronal migration; cerebellum; foliation

Abbreviations: external granular layer (EGL); molecular layer (ML); Purkinje cell layer (PCL); internal granular layer (IGL); white matter (WM); proliferative zone (PRZ); premigratory zone (PMZ); metronidazole-induced encephalopathy (MIE); mother body weight (MBW); body weight change during pregnancy (ΔMBW); pups' body weight on birth (FBW); FM-fetuses with malformation; fetus/mother metronidazole serum concentration ratio (F/M Mtz-average); pial membrane (PM); high performance liquid chromatography (HPLC); hematoxylin and eosin (H&E).

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INTRODUCTION

The most vulnerable age for the central nervous system is the period of neuronal division, migration and differentiation. The first findings of neuronal migration based on neuronal-glial interaction were described in a cerebellar development model [1]. Due to its specific structure, the cerebellum has so far been the subject of numerous studies examining the neurotoxic potential of different chemicals [2-5], ionizing radiation [6], as well as deficiencies of specific nutritional factors [7,8].

Metronidazole is a widely used drug in the treatment of amoebiasis, protozoal infections, vaginosis and nonspecific vaginitis [9]. However, its use during pregnancy still occupies many divided views among gynecologists [10]. Its neurotoxic potential stands out as one frequent side effect. The cerebellar dysfunction, peripheral neuropathy, encephalopathy, as well as epileptic seizures in adults, represent a unique entity: metronidazole-induced encephalopathy [12]. The small number of experimental studies that have examined the use of metronidazole during the prenatal period of life have revealed that the administration of high doses of metronidazole produce teratogenic and fetotoxic effect [13, 14].

The use of guinea pigs as an experimental model of cerebellar development affords certain advantages over the commonly used laboratory rodent species [15-17]. Practically complete foliation in guinea pigs takes place prenatally, unlike in mice and rats where it begins on the last day of intrauterine life [18]. The fact that the last third of gestation in guinea pigs developmentally corresponds to the third trimester in humans [19] led us to choose this animal model. Our study aimed to examine the effects of prenatal treatment with metronidazole on the process of cerebellar folia development in guinea pig fetuses.

MATERIALS AND METHODS

Animals

Albino guinea pigs (*Cavia porcellus*), aged between 4 and 5 months, obtained from the Animal Facility of Military Academy Belgrade, Belgrade, Serbia, were studied. The experiments were approved by the Ethics Committee for Animal Research of the University of Novi Sad, Serbia (No: 01-131/3-0). The animals were kept in 400 mm (wide) x 1000 mm (long) x 300 mm (high) plastic containers in a harem system, i.e. with 3 or 4 females per male. Artificial cycles with 12 h of light (08:00-20:00) and 12 h of dark were used. The ambient temperature was 23°C and the air was fully circulated 6 to 10 times per h. Inspection of the female vaginal introitus was performed daily. The presence of spermatozoids in the vaginal smear was designated as the first day of gestation when the dams were housed separately from males. Gestational length in guinea pigs can vary between 62 and 68 days [20]. The experimental study included 12 female animals that maintained pregnancy until they were euthanized. These were randomly divided into two groups: the control group, C (n=6) and the experimental group, E (n=6).

Experimental design

The 8-day treatment began on the 42nd and finished on the 49th day of gestation for both groups of dams. Metronidazole (Sigma-Aldrich, Germany) used in this study was dissolved in a 0.9% saline solution (Hemofarm, Serbia). The dose of metronidazole was calculated using the Food and Drug Administration's Human Equivalent Dose formula [21], and the applied experimental protocol simulated a procedure of vaginal infection treatment during pregnancy [22]. The E group received a subcutaneous injection of metronidazole solution at a dose of 28 mg/kg at 8 a.m. and 8 p.m. The C group received saline solution in the same volume and time protocol. On the 50th day of gestation (at 8 a.m.), the fetuses (19 in the E, and 20 in the C group) were removed by Cesarean section under urethane anesthesia (1.0 g/kg) and then weighed. Cardiopuncture was performed to collect the blood and the whole body was fixed by perfusion fixation protocol previously described [7]. After fixation, the central nervous structures (cerebrum, cerebellum, medulla oblongata and spinal cord) were removed and immersed in Zamboni fixative for 24 h at 4°C. For this study, the data derived from the cerebellum are only presented.

Tissue processing and immunohistochemistry

After photographing the gross changes, a 5-mm thick midsagittal section of the vermal region of the cer-

ebellum was taken. The slides were dehydrated in isopropyl alcohol and embedded in paraffin (Histowax, Netherlands) and cut on a rotary microtome (Leica, Germany) in 5-μm sections. The sections were stained with hematoxylin and eosin (H&E) and by immunohistochemical staining, including primary antibodies: rabbit anti-neuronal nuclear (NeuN) antigen at a 1:500 dilution (Abcam, UK), rabbit anti-proliferating cell nuclear (PCNA) antigen at a 1:500 dilution (Abcam, UK), rabbit anti-Caspase 3 at a 1:150 dilution (Cell Signaling, USA), rabbit anti-microtubule-associated protein 2 (MAP2) at a 1:8000 dilution (Abcam, UK), rabbit anti-S100 at a 1:1000 dilution (Abcam, UK), rabbit anti-calbindin D-28K at a 1:10000 dilution (Swant, Switzerland), and the visualization system using a mouse and rabbit-specific HRP/DAB (ABC) detection IHC kit (Abcam, UK). All the antibodies were applied for 60 min at room temperature. Before application, sections were subjected to antigen retrieval pretreatment (except calbindin) using citrate buffer (pH 6.0) in a microwave oven at 850 W for 20 min. Visualization was performed using the DAB Substrate Kit (Abcam, UK). Mayer's hematoxylin was used as a counterstain for immunohistochemistry.

HPLC of metronidazole blood serum concentration

All reagents and solutions were either high performance liquid chromatography (HPLC) or analytical grades. Metronidazole was obtained from Sigma (UK), methanol and water were from JT Baker (USA). For protein precipitation, 500 μL of methanol was added to 200 μL of plasma in a 3-mL test tube and the samples were vortexed, followed by centrifugation at 3000 x g for 10 min. The supernatant layer was separated, of which 10 μL was injected onto the column and the peak areas were recorded. A reversed-phase HPLC method was used to determine metronidazole plasma concentrations [21] with the Dionex USA HPLC apparatus. Chromatographic separation was performed using an ODS Hypersil (Agilent, 150 mm x 2.1 mm, 5 μm) column, and the ODS (Agilent; 20 mm x 2.1 mm, 5 μm) guard column. The mobile phase consisted of 5% methanol in 0.05 M of KH_2PO_4 buffer (pH=4.0). The aqueous phase was eluted at a flow rate of 0.5 mL/min and the effluent was monitored at 254 nm [23].

Morphometric analysis of the folia

All histology slides were scanned using a VisionTek® (Sakura, Japan) digital microscope under 100× magnifications and saved in digital form. The lobule nomenclature of the vermis used here is based on the anatomical atlas of the guinea pig of Cooper and Schiller [15]. It included the sagittal section of the cerebellar vermis subdivision of the primary lobes of Larsell [24] as follows: lobules I-III (anterobasal lobe), IV-V (anterodorsal lobe), VI-VIII (central), IX (posterior) and X (inferior) [24]. Morphometric analysis was performed on H&E digitalized slides using image tool-free computer software. Using the plug-in area, we measured the areal fraction of each folium and the external granular layer (EGL), the molecular layer (ML), the Purkinje cell layer (PCL), the internal granular layer (IGL) and the white matter (WM). All results were represented graphically as a mean value.

Statistical analysis

Statistical analysis was performed using IBM SPSS statistical software, ver. 19.0 (IBM Corp., Armonk, NY, USA). The data were reported as the mean±standard deviation (SD) and assessed for normality using the Kolmogorov-Smirnov test. An independent-samples t-test or Mann-Whitney U test as a nonparametric equivalent was used to compare groups. Mixed between-within subject analysis of variance (ANOVA) was conducted to estimate the effect of time and treatment in the experimental animals. Pearson's product-moment correlation coefficient was used to explore relationships among variables. A difference between groups was considered statistically significant for a *P*-value less than 0.05 ($P<0.05$).

RESULTS

Detection of metronidazole in blood serum and its impact on the body weight of mothers and fetuses

A mixed between-within subjects ANOVA was conducted to assess the impact of metronidazole and saline treatment on body-weight change in guinea pig mothers during three time periods (before interven-

Table 1. Body weight of mothers and fetuses, number of fetuses with malformations and the average metronidazole fetus:mother serum concentration ratio in the control and experimental groups.

Mother group[1]	MBW 1	MBW 5	MBW 9	ΔMBW	FBW	FM	F/M Mtz
Control	709.5±56.9	749.7±58.3	786.7±62.8	78.6±37.5	33.7±1.2	0/20	0+0
Experimental	758.3±96.7	780.8±118.2	803.0±110.2	44.7±30.6	30.8±2.3*	12/19	0.88±0.06

[1]Each mother group was comprised of 6 female guinea pigs, * $P<0.05$; MBW – mother body weight; MBW – body weight change during pregnancy; FBW – pup body weight; FM – fetuses with malformations; F/M Mtz – average fetus/mother metronidazole serum concentration ratio

tion – MBW1, 5th day of intervention – MBW5 and the day of euthanasia – MBW9). There was no significant interaction between treatment type and time (Wilks' Lambda=0.761, F(2, 9)=1.416, P=0.292, partial eta squared=0.239). There was a substantial main effect for time (Wilks' Lambda=0.184, F(2, 9)=19.960, P<0.01, partial eta squared=0.816), with both groups showing an increase in body weight across the three time periods. The main effect of the two types of intervention was not significant (F(1, 10)=0.419, P=0.532, partial eta squared=0.040), suggesting no difference in the effect of the two interventions on the body weight of guinea pig mothers (Table 1).

An independent-samples t-test was conducted to compare the body weight change for the two groups of guinea pig mothers and to compare the body weights of the pups at birth (Table 1). There was no significant difference in body-weight change between the control vs the experimental group (78.6±37.5 *vs* 44.7±30.6; t(10)=1.734, P=0.114, two-tailed). There was a significant difference in the body weight of the pups at birth (t(10)=2.655, P<0.05, two-tailed)). The magnitude of the differences in the means (mean difference=2.833, 95% CI: 0.455 to 5.211) was large (eta squared=0.176). Furthermore, there were no pups with malformations in the C group, while in the E group 12 out of 19 pups had malformations (Table 1). Finally, metronidazole achieved high concentrations in pup serum, with an average fetus/mother serum concentration ratio of 0.88±0.06 (14.46±1.08/16.41±1.48 μg/mL) (Table 1).

The relationship between the body weights and metronidazole concentrations of guinea pig mothers and pups was investigated using Pearson's product-moment correlation coefficient. Preliminary analyses were performed to ensure no violation of the assumptions of normality, linearity and homoscedasticity. There was a strong negative correlation between the body weight of the pups and metronidazole concentration in mothers (r=–0.641, P<0.05), a strong negative correlation between the body weight of the pups and the concentration of metronidazole in pups (r=–0.612, P<0.05), and a strong positive correlation between metronidazole concentration in mothers and metronidazole concentration in pups (r=0.995, P<0.001).

Gross changes in the cerebellum of metronidazole-treated fetuses

Gross analysis of the vermal region of the cerebellum in all 20 fetuses from the C group showed the appropriate number and correct arrangement of the folia. Each folium and subfolium had regular connections with the paravermal region and were in continuity with the lobes of the laterally located hemispheres (Fig. 1A). On

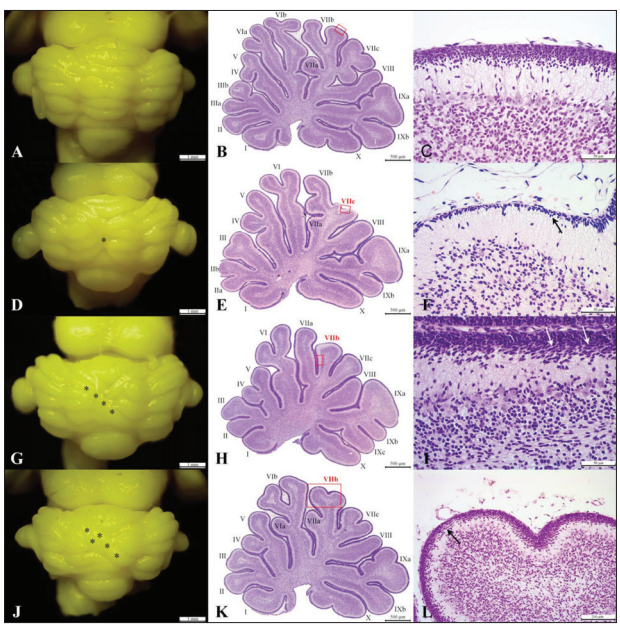

Fig. 1. Gross and histological features of the cerebellum in control (**A**, **B**, **C**) and experimental (**D**-**L**) animals on the 50th day of gestation. **A** – The number and arrangement of the folia in the control group of animals. The asterisks point to irregular depression (**D**) and malposition of the folia (**D**, **G**, **J**); **B**, **E**, **H**, **K** – H&E staining – 10×; **C**, **F**, **I** – 200×; **L** – 100×. Black and white arrows point to the granular cell depletion and nuclear reorientation, respectively. Scale bar represents 1 mm (**A**, **D**, **G**, **J**), 500 μm (**B**, **E**, **H**, **K**), 200 μm (**L**) and 50 μm (**C**, **F**, **I**).

the other hand, in the E group, in 12 out of 19 fetuses (63%), a macroscopically visible disorder of the cerebellar folia was observed. Changes predominantly found in the dorsal lobe of the vermis were characterized by the existence of irregular depressions localized in the central line of the vermis (Fig. 1D, asterisk) as well as frequent malposition of the folia (Fig. 1G, J asterisk). This depression was characterized by a clear break in the continuity of the folia. However, the relationship with the paravermal region was still preserved (Fig. 1D, G). In all cases, abnormalities were predominantly observed in folia VII and associated subfolia (VII a-c) (Fig. 1E, H, K).

Qualitative pathological changes of the cerebellar cortex in metronidazole-treated fetuses

The sagittal section of the vermal region of the cerebellum in group C exhibited the existence of all ten cerebellar lobules with the most common additional sub-segmentation of lobules III to IIIa+IIIb, VI to VIa+VIb, VII to VIIa+VIIb+VIIc and IX to XIa+XIb (Fig. 1B). Histologically, the EGL composed of the proliferative (PRZ) and premigratory (PMZ) zone was observed subpially. At the sagittal cross-section, the nuclei of EGL neurons in both zones assumed the expected circular shape (Fig. 1C). PCNA-positive neurons of the PRZ (Fig. 2B) and NeuN positive postmitotic neurons of the PMZ (Fig. 2A) were identified. The ML was well developed with visible granular cells in a radial migration process (Fig. 2A), as well as rich dendritic arborization of Purkinje cells positive on Calbindin D-28K (Fig. 2D) and MAP2 markers (Fig. 2E). This planar dendritic plexus of Purkinje cell is always oriented perpendicular to the long axis of the folium, which is referred to as a translobular plane. Immediately below the PN was the primarily formed IGL composed of small NeuN positive postmitotic granular neurons (Fig. 2A). Visualization of Bergmann glia with the S100 marker showed regularity in their number and orientation (Fig. 2F).

In the E group with macroscopically observed cerebellar malformations, histological changes were also evident. This change was predominantly present in folia VII of the cerebellum. The macroscopic irregular depression displayed histologically manifested atrophy of all cerebellar cortex layers (Fig. 1E). Accordingly,

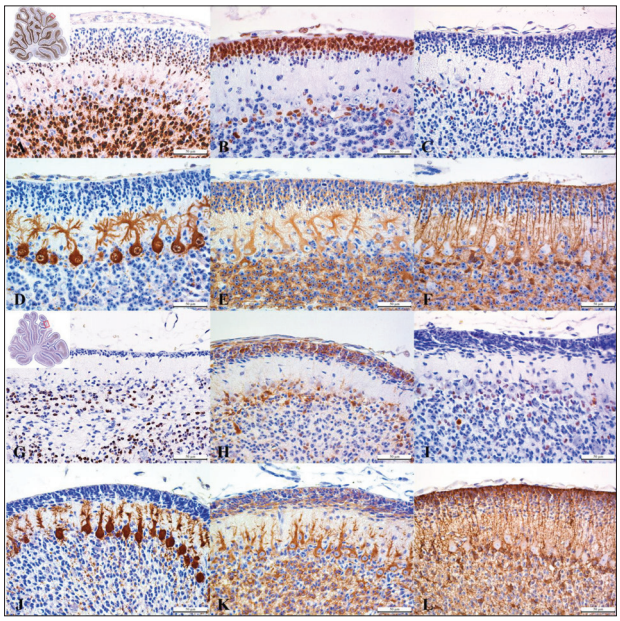

Fig. 2. Immunohistochemical staining of the cerebellar cortex in the control (**A**-**F**) and experimental (**G**-**L**) groups of animals; anti-NeuN (**A**, **G**), anti-PCNA (**B**, **H**), anti-caspase 3 (**C**, **I**), anti-calbindin D-28K (**D**, **J**), anti-MAP2 (**E**, **K**), anti-S100 (**F**, **L**). Scale bar represents 50 μm (**A**-**L**).

the EGL was reduced, with only 2 to 3 layers of NeuN-negative granular cells (Fig. 1F, Fig. 2G). The ML was extensively thinned, with a reduced planar dendritic plexus of Purkinje cell. The IGL was also reduced, with rare NeuN-positive neurons (Fig. 2G). Histological analysis of the folia malposition showed changes in the EGL and PCL. Namely, although the PRZ was preserved (Fig. 2H), the PMZ was characterized by an unusual longitudinal orientation of granulosa cell nuclei (Fig. 1I, L). At the same time, the Purkinje cells exhibited the absence of rich dendritic arborization, which was represented by only one primary dendrite (Fig. 2J, K). This cytoarchitectonic organization of the EGL and PCL is typically found at the coronal projection of the cerebellar vermis but not in the sagittal. The weakening of the glial fibrillary acidic protein (GFAP) immunopositivity in Bergman's glial cell was also detected (Fig. 2L). No significant differences in the distribution of caspase 3 immunopositivity were observed between the E (Fig. 2I) and C groups of fetuses (Fig. 2C). The changes in the cortical layers and the folia disturbance are presented in the schematic line drawing of the vermis in the E group (Fig. 3C, D), and its preservation in the C group (Fig. 3A, B).

Quantification and distribution of the pathological changes in the cerebellar cortex in metronidazole-treated fetuses

The morphometric analysis of the areal fraction of each folium revealed extensive changes in the E as compared to the C group of fetuses. A statistical decrease in the areal fraction was found in all folia, with $P<0.05$ for folia II and IV, and $P<0.01$ for the remaining folia (Fig. 4A). Segmental analysis of the areal fraction in the EGL revealed a decreasing of $P<0.05$ in folia V and VI, and $P<0.01$ in folia I, VII, VIII, IX and X (Fig. 4B). Also, a decrease of the areal fraction in the ML was detected in all folia, with $P<0.05$ in folia II and IV, and $P<0.01$ for the remaining folia (Fig. 4C). As an unavoidable consequence of the changes in the EGL, we detected an intense decrease in the areal fraction in the IGL, with $P<0.05$ in folia I, III, V, VI, VII, VIII, IX and X (Fig. 4D). Changes in the WM were detected only in folia VII ($P<0.01$) and IX ($P< 0.05$) (Fig. 4E).

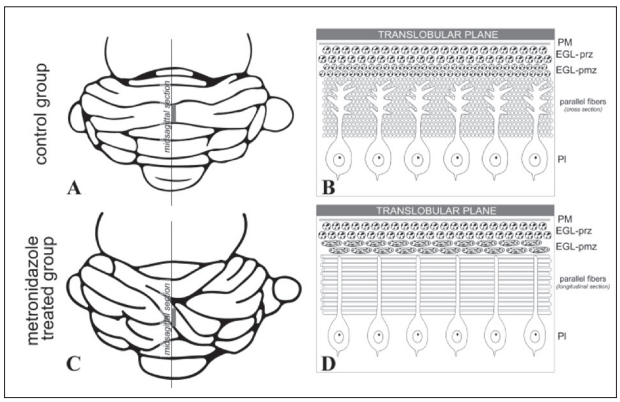

Fig. 3. Line drawings of the gross dorsal view of the vermis in the control (**A**) and the metronidazole-treated, experimental group (**C**). Schematic drawing of the cerebellar cortex from the midsagittal section of the vermal region. PM – pial membrane; EGL-prz – external granular layer – proliferative zone; EGL-pmz: external granular layer – premigratory zone; PCL – Purkinje cell layer.

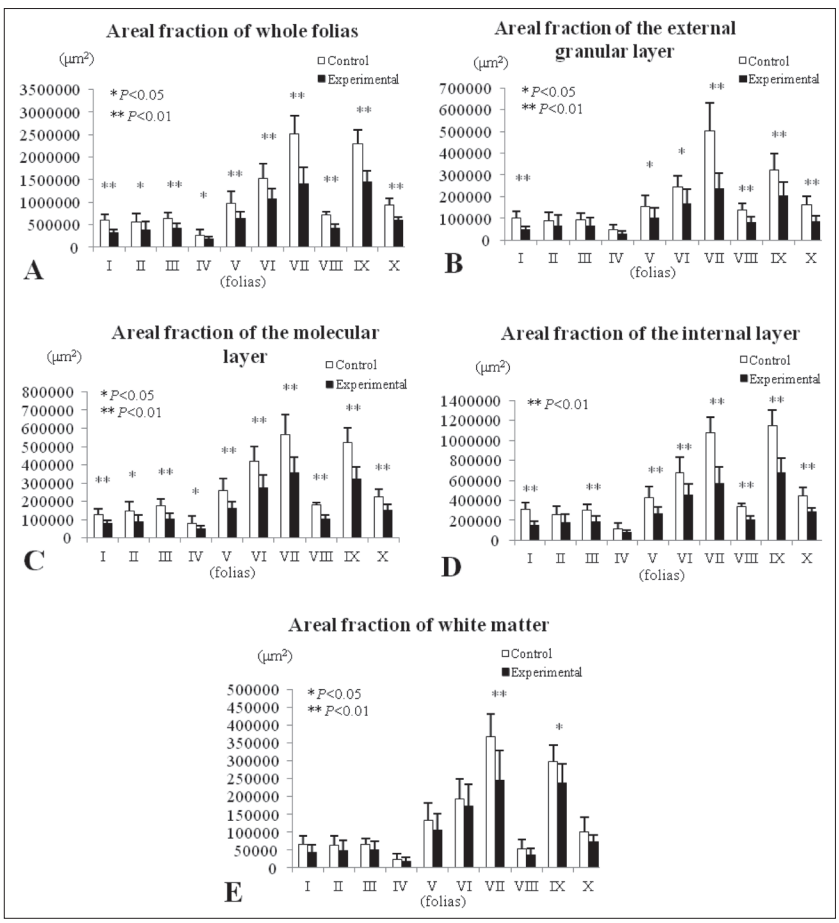

Fig. 4. Quantification and distribution of pathological changes of the cerebellar cortex. Areal fraction analysis in whole folia (**A**) and each layer: external granular layer (**B**), molecular layer (**C**), internal granular layer (**D**) and white matter (**E**). Results are presented as the means±SD (n=20 specimens per control group; n=19 specimens per experimental group); *$P<0.05$ indicates a significant difference between experimental and control animals; **$P<0.01$ indicates a high significant difference between experimental and control animals.

DISCUSSION

Regularity of the cellular organization of the central nervous system directly depends on the process of neural migration as the primary mechanism of brain development [25-27]. Various chemicals and mutations are just some of the agents that lead to the disturbance in cortical development [28-30], although the true etiopathogenesis is usually unclear [31]. Despite numerous limitations of animal models, the use of guinea pigs in research and the level of translation of the obtained results to humans have found extensive application, especially in reproductive studies [19]. Unlike other rodents, in guinea pigs brain development occurs mainly prenatally, which is closer to the human model of development [15-17,32]. Studies indicate that the last third of gestation in guinea pigs directly corresponds to the last trimester in

humans [19]. Also, guinea pigs belong to precocial species with a long gestation period (about 65 days), giving birth to mature pups, while other rodents (rat, mouse, hamster, etc.) belong to altricial animals that give birth to immature pups [15]. Long gestation with prenatal brain development has positioned guinea pig use ahead of the more commonly used rodents in comparative prenatal development studies [32].

The first case of metronidazole-induced encephalopathy (MIE) was recorded in 1959 [32-34]. MIE is characterized by cervical dysfunction, altered mental status, as well as epileptic seizures [12]. Although metronidazole officially belongs to the B group of drugs for safety in pregnancy [9], its use still provokes divided opinion among physicians. Most do not recommend it in the first trimester of pregnancy, while its use in the 2nd and 3rd trimesters is justified only in cases where alternative therapy is unsuccessful [10].

Experimental studies performed on rats [36] and dogs [37] confirmed the high neurotoxic potential of metronidazole, mainly characterized by degenerative changes in the brain that were localized in the cerebellar region. However, a small number of experiments in which the prenatal effect of this drug was examined stand out. The combination of metronidazole and alcohol, administrated during the prenatal period, showed a high fetotoxic and teratogenic potential. Namely, malformations such as cataracts, microphthalmia, exencephaly, renal atrophy, hydroureter and cleft palate were observed in 3.6% of metronidazole-treated fetuses [38]. A recent study of prenatal administration of metronidazole alone and in combination with miconazole during early gestation in mice reported the appearance of skeletal malformations [39]. Listed malformations and the absence of foliation disturbance, as found in our research, could be explained by the postnatal appearance of this process in mice.

The foliation itself and the final fixed number of 10 folia point to the genetic basis of this process in which the complexity and number of subsegments increases in evolutionarily higher mammalian species [24,40]. Several chemicals [2-5], radiation [6], the absence of nutritional factors [7,8] as well as genetic mutations [41,42] are linked to the disturbance in the cerebellar cortex and folia development. Changes such as hypofissure, malposition and agenesis of the folia usually dominate. We also observed similar changes in our research. The microscopic changes that dominated in the altered folia were atrophy of the EGL and ML accompanied by disturbance of the orientation of parallel fibers and the dendritic plexus of Purkinje cells [2,3]. Almost the same histological pattern was observed in our study. Namely, regardless of the etiological factor, the EGL and its PRZ represent the most vulnerable zone in the cerebellum. Loss of granular cells or disturbance in their polarity in the PMZ directly lead to the reorientation of parallel fibers and folia direction [2]. Although parallel fibers were not identified in our study, the appearance of a longitudinal orientation of the cells of the PMZ, with atrophy of the ML in the E group, explains the identified cortical and folia disturbances. Changes in the EGL and IGL almost inevitably have repercussions on the development of Purkinje cells and the ML [43]. This phenomenon is defined by the orthogonal organization of practically all neural and glial components of the cerebellar cortex. Purkinje cells, ML interneurons as well as climbing fibers, are oriented within the translobular-parasagittal plane, while parallel fibers and Bergman's glia are oriented within the parlobular-coronary plane [40,42]. Any change in the cytoarchitectural orientation, such as the position of parallel fibers, will directly affect the development and presentation of the dendritic tree of Purkinje neurons [44,45]. Thus, we hypothesized that metronidazole-induced loss or changes in the polarization of granular cells in the EGL caused the reorientation of parallel fibers, with changes in the Purkinje cells' tree. All these microscopic changes directly correlate with the difference in the direction of the folia, i.e. the appearance of malposition.

Although several hypotheses have been put forward, the precise neurotoxic mechanism of metronidazole is still not fully defined. One of the potential mechanisms is the binding of metronidazole intermediates to neural DNA and RNA and consequent inhibition of protein synthesis [46,47]. In our study, given the apparent impairment of the EGL in which the processes of cell division and protein synthesis take place, this mechanism can undoubtedly be closely correlated with the obtained results.

Recent *in vitro* studies that analyzed mitochondrial function, oxidative stress and cytotoxicity indicated that metronidazole does not affect the production of

reactive oxygen species (ROS), which suggests that the mechanism of neuronal death is most likely mediated by a ROS-independent mechanism [48]. Postnatal administration of phenytoin to 5-day-old rats leads to the induction of EGL cell apoptosis and disruption of their migration [4].

In our study, caspase 3 staining did not reveal a proapoptotic pathological effect of metronidazole. The distribution of positivity for this marker was equally present in both study groups. It is important to note that apoptosis and proliferation are generally present during migration and maturation of neurons as an indispensable physiological mechanism of neural development [15]. Although diffuse atrophy of the cerebellar cortex was confirmed by morphometric analysis, folia VII stands out with its abundant macroscopic and microscopic changes. This phenomenon could be explained by the fact that cerebellar folia develop within three consecutive temporal neurogenetic divisions. The first, designated as the early generated division, involves the formation of I, X, and in part folia II. The 2nd, designated as an intermediate-generated division, involves the formation of parts of folia II, III, IV, V and IX. The last, 3rd late-generated division involves the formation of folia VI, VII and VIII [49]. Given the very scarce data on the dynamics of cerebellar foliation in guinea pigs based on Larsell's classification [24], we performed a preliminary study of guinea pig cerebellar foliation. The first signs of foliation in guinea pigs were detected on the 35th day of intrauterine life, and the finalization of this process was from the 40th to the 50th intrauterine day. By classifying folia VII as a late-generated division, it is clear why the observed changes were most intense during this stage.

The safety of metronidazole in pregnancy is still a matter of discussion among clinicians. Most of the studies indicate that the prenatal use of metronidazole leads to a decrease in fetal growth and an increase in malformation incidence [50-54]; however, there are still some studies that point to its teratogenic and mutagenic potential [55-58]. On the other hand, the mentioned studies did not focus on changes in cerebellar development. Folia aberration and cortical disturbance, as described in our research, open a new aspect of potential reconsideration of the safety of metronidazole. Recent studies indicated that a similar disturbance in cerebellar migration leads to functional deficits that are clinically presented as autism, epilepsy and cognitive defects [59,60].

CONCLUSION

The use of guinea pigs as an animal model of neural developmental disorders revealed its potential in the present study. Metronidazole induced selective depletion of proliferative zones in the cerebellum and a considerable decrease of the areal fraction in all cortical layers. The subsequent reorientation of Purkinje and granular cells caused an alteration of the folia. The presented results represent a new aspect of prenatal neurotoxic side effects of metronidazole. We hope that these results will promote a reevaluation of the use of this drug in pregnancy.

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Conflict of interest disclosure: The authors declare no conflict of interest.

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