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Cerebellar Toxicity Induced by Maternal Exposure to Topical Betamethasone Prior to Fertilization in Newborn Rabbits

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ABSTRACT

Background: Betamethasone is a synthetic glucocorticoid used for treatment of Psoriasis. Until now, the side effect of maternal exposure to topical betamethasone, prior to fertilization, on newborn is not clear. So, the aim of the present study was to determine this side effect.

Methods: We used two doses of betamethasone: 0.02 and 0.2 mg/kg b.w. At the end of the experimental period (after 3 weeks of delivery), six new-born rabbits of both sexes (1 newborn rabbit per mother) were dissected under light anesthesia and blood and cerebellum samples were collected. All the measurements were performed in one assay.

Result: The results of this study revealed that betamethasone can bioaccumulate in sera of rabbits treated mothers. Maternal exposure to betamethasone, for 2 months before fertilization, induced oxidative stress, alterations in levels of studied neurotransmitters and increase in activities of acetylcholinesterase and caspase-3 enzymes. Furthermore, these biochemical alterations were supported by histopathological observations. So, this study suggested that females; desiring to be pregnant, should stop treatment of psoriasis with betamethasone before fertilization to avoid cerebellar toxicity of their offspring.

Key words: Betamethasone, Cerebellum, Maternal exposure, Newborn, Psoriasis.

INTRODUCTION

Topical glucocorticoids are used for treatment of psoriasis, mainly due to their immunosuppressive, anti-inflammatory and antiproliferative properties (Castela *et al.*, 2012) as endogenous biosynthesis of glucocorticoids is suppressed in psoriatic skin (Sarkar *et al.*, 2017).

Endogenous and synthetic glucocorticoids regulate epidermal development and combat skin inflammatory diseases (Bigas *et al.*, 2018). So, glucocorticoids are considered as the first line treatment for all grades of psoriasis (Laws and Roung, 2010). Betamethasone is more effective than any other steroids (Ni *et al.*, 20l8). It is well known that the highest level of glucocorticoid receptors is present in the cerebellum, so cerebellum is an important area of study (Pavlik *et al.*, 1984).

Glucocorticoids (GC) act throughout the body *via* the widely expressed glucocorticoid receptor and the mineralocorticoid receptor (Pujols *et al.*, 2002). GC binding to its receptors results in a wide array of genomic and non-genomic signaling changes at the cellular level (Kadmiel and Cidlowski, 2013).

Corticosteroids have widespread pharmacological effects mediated *via* gene transcription and protein expression effects in many organ systems. This leads to both desired fetal effects such as increased protein production in the lungs with resulting phospholipid biosynthesis and the appearance of surfactant (Ballard and Ballard, 1995), potentially less desirable effects such as decreased cerebral blood flow that could be linked to adverse neurodevelopment (Schwab *et al.,* 2000).

In this regard, some animal and human-based studies could confirm the beneficial effects of corticosteroids, but some others did not recommend such regimens due to their related adverse consequences (Arimi *et al.,* 2021). There is considerable evidence from experimental animals that corticosteroids can have an adverse effect on the growth and development of the immature brain (Whitelawa and Thoresen, 2000).

Prenatal glucocorticoids increase the susceptibility of cerebellar neurons to oxidative cell death (Fuentes-Pardo *et al.*, 1990) and glucocorticoids therapy may disrupt cerebellar development through the rapid induction of apoptosis (Noguchi, 2014). Hypercorticosteronemia induced brain region-specific changes that might include aberrant myelination and a degree of white matter damage (Amya *et al.*, 2021).

Glucocorticoids play a pivotal role in fetal programming (Czamara *et al.*, 2021). Although, there are no enough studies about the risk or potential alterations due to accumulation of Betamethasone before fertilization. In

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addition, previous studies paid attention to intramuscular injection during pregnancy neglecting the long-term impact of the use of topical solution. So, the goal of the present study was to determine these adverse side effects topical administration of betamethasone solution on liver and kidney tissues.

Therefore, the goal of the present study was to establish whether maternal topical exposure to betamethasone, 2 months before fertilization, would induce alterations in new born rabbits.

MATERIALS AND METHODS

Experimental animals

Twenty-four adult healthy sexual mature, White New Zeeland rabbits *Oryctolagus cuniculus* of both sexes (6 males and 18 females) of average weight of 2.5±0.5 kg, 6-8 months obtained from research station in king Faisal University. The animals were housed in steel cages under controlled condition like temperature 25±2°C and 12 hrs. light /dark cycle with free access to water, labium and pellet food diets. They were left for two weeks for acclimatization before starting the experiment. All experimental procedures were reviewed and approved by the research ethics committee at King Faisal University (Ref. No. KFU-REC/2021-03-05). They were housed in the animal house of College of Science, Building 9, King Faisal University.

Experimental design

The experiment involved three experimental groups as follows:

G I (Control group)

In this group, female rabbits (n=6) were topically exposed on the shaved area of back skin to the saline solution for 2 months and allowed to mate with males and then kept until delivery.

G II

In this group, female rabbits (n=6) were topically treated with betamethasone solution at a dose of 0.02 mg/kg body weight on the shaved area of back skin for 2 months, allowed to mate with males and then kept until delivery.

G III

In this group, female rabbits (n=6) were treated with betamethasone solution at a dose of 0.20 mg/kg body weight on the shaved area of back skin for 2 months, allowed to mate with males and then kept until delivery.

Mating

Mating process was done according to the method of Wangikar *et al*., (2005).

Application of medication

Betnovate solution - Scalp application 30 ml -Betamethasone 0.1% w/w (as Betamethasone Valerate) was topically applied on shaving area of back skin (5 × 5), for 2 months according to Vose *et al.* (2014).

At the end of the experimental period (after 3 weeks of delivery), six newborn rabbits of both sexes (1 newborn rabbit per mother) were dissected under light anesthesia. The animals were euthanized anesthetic exsanguination using the combination of 10 mg/kg xylazine and 100 mg/kg ketamine HCl. The blood samples (5 ml) were collected aseptically through vein puncture from lateral cephalic vein of each rabbit. Cerebellar tissues were collected and kept frozen at -80°C before assays for 24 hours. Collected cerebellar tissue samples were used for biochemical and histological evaluations. All the measurements were performed in one assay.

Determination of bioaccumulation of betametathone in sera of rabbits mothers treated with betamethasone (2 monthes before fertilization)

Evaluation of betamethasone concentrations in the blood of female rabbits, treated with betamethasone for 2 months before fertilization, were done every 7 days during the period of treatment using commercial REAGEN betamethasone ELISA Test Kit Manual, which is a competitive enzyme immunoassay. The concentration of betamethasone was determined as ng/ml.

Determination of antioxidant markers

Activities of catalase, superoxide dismutase and glutathione peroxidase were determined in chosen tissues using commercial kits.

Determination of acetylcholinesterase activity

The activity of acetylcholinesterase enzyme was performed using AmpliteTM colorimetric acetylcholinesterase assay kit according to the instructions of the supplier.

Estimation of GABA level

The level of GABA was determined as described in the manual of Rat Gamma-aminobutyric acid, GABA ELISA Kit. GABA level was calculated as mg/ml/g Tissue.

Determination of serotonin and dopamine levels

Serotonin and Dopamine levels were measured using competitive ELISA kits. They were calculated as ng/mg protein.

Light microscopic study

Cerebellar specimens were collected from all experimental groups and fixed in 10% neutral buffered formalin and processed routinely to be stained with Haematoxylin and Eosin stain (H&E). Tissue blocks were cut into thin section 5 microns and stained with HandE stain (Bancroft and Gamble, 2002). The stained sections were examined using light microscopy.

Statistical analysis

Results were presented as mean±standard error of the mean (SE). SPSS program was used for the statistical analysis of data with one-way ANOVA to compare the experimental groups. A difference was considered significant when $P \leq 0.05$.

RESULTS AND DISCUSSION

Bioaccumulation of betametathone in sera of rabbits mothers treated with betamethasone (2 monthes before fertilization)

In the present study (Table 1) the exposure to betamethasone, for 2 months before fertilization during 8 weeks, resulted in bioaccumulation of betamethasone in sera of treated rabbits. It resulted to approximately 16-fold increase in low dose betamethasone treated group (from 0.63 to 3.85 ng/ml) and high dose betamethasone treated group (from 0.78 to 4.87).

Effect of maternal betamethasone exposure before fertilization on oxidative stress markers in newborn rabbits

In the present study (Table 2), new born rabbits of G II and G III, showed significant decrease in studied antioxidant enzymes activities and increase in the MDA levels in cerebellum tissues as (G I). These alterations were more pronounced in rabbits of GIII than those of G II. These can be considered as indicators of oxidative stress. It was reported that prenatal or postnatal glucocorticoid therapy induced oxidative stress in cerebellar granular cells (Ahlbom *et al.*, 2000) or in developing brain in rats (Camm *et al.*, 2011) respectively.

Effect of maternal betamethasone exposure before fertilization on some neurotransmitter levels and acetylcholinesterase and caspase 3 activities in cerebella of newborn rabbits

In this study, there was a significant increase in acetylcholinesterase and caspace-3 activities and GABA level but a significant decrease in the concentrations of serotonin and dopamine (Table 3) in newborn rabbits, whose mothers were exposed to betamethasone before fertilization for 2 months (G II and G III) relative to control group (G I). This result is in agreement with the finding of Yamate *et al.* (2010) who reported that administration of betamethasone caused higher level of acetylcholinesterase activity in cerebellum of chick embryos (19 days of age) than control. Serotonin acts as a differentiation factor in early neurogenesis. So, change in serotonin level during brain development altered neuronal differentiation (Buznikoy, 1984). A hypothesis is that glucocorticoids regulate the development of mechanisms which couple neuronal depolarization with release of neurotransmitter (Puro, 1983). The results of Kawamura *et al.* (1984) suggest that adrenal glucocorticoids may directly act on brain serotonin metabolism and there may be a feed-back relation between adrenal glucocorticoid and brain serotonin.

Alteration in dopamine level, in this study, may indicate alteration in normal fetuses' development. This can be supported by the fact that postnatal exposure to glucocorticoids led to preterm cerebellar growth impairment (Tam *et al.*, 2011). In this study, maternal administration of betamethasone (0.02 mg/kg or 0.20 mg/kg) for 2 months before fertilization showed significant increase in GABA level and significant decrease in serotonin and dopamine levels in cerebellum of new born rabbits which may suggest the ability of betamethasone to alter neurotransmitter levels in these newborn rabbits. These results are in agreement with Wyroll and Holmes (2011). Also, it is well known that glucocorticoids facilitate synaptic GABA release (Di *et al.*, 2009).

Apoptosis through caspase-mediated mechanisms play a role in brain injury (Gill and Perez-Polo, 2008). This study demonstrated that maternal betamethasone exposure before fertilization increased caspase 3 activity in cerebellum of new born rabbits. This may suggest that Betamethasone

Table 1: Showing bioaccumulation of betamethasone in sera of of rabbit's mothers treated with betamethasone (2 monthes before fertilization).

	No.	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Group I	6	0.01^{c}±0.0	0.01^{c}±0.0	0.01^{c}±0.0	0.01^{c}±0.0	0.01^{c}±0.0	0.01^{c}±0.0	0.01^{c}±0.0	0.01^{c}±0.0
Group II	6	0.63^{b}±0.007	0.83^{b}±0.003	1.04^{b}±0.003	2.13^{b}±0.048	2.65^{b}±0.004	2.94^{b}±0.004	3.01^{b}±0.004	3.85^{b}±0.004
Group III	6	0.78^{a}±0.004	0.87^{a}±0.006	1.55^{a}±0.009	2.85^{a}±0.006	3.52^{a}±0.009	4.24^{a}±0.087	4.35^{a}±0.006	4.87^{a}±0.016
F (p)		8113.768* (<0.001*)	13968.500* (<0.001*)	20113.364* (<0.001*)	2798.206* (<0.001*)	100233.0* (<0.001*)	1865.417* (<0.001*)	263136.111* (<0.001*)	76155.194* (<0.001*)

Data are presented as means±SE. N=Six experimental animals per group.

Mean values with similar letters are insignificant. *Statistically significant at P≤0.05.

Table 2: Effect of maternal betamethasone exposure before fertilization on oxidative stress markers in cerebellum of newborn rabbits.

	No.	Cerebellum			
		CATU/g tissue	SODU/g tissue	GPXU/g tissue	MDA nmol/g tissue
Group I	6	42.6^{a}±0.7	61.9^{a}±0.5	65.6^{a}±0.9	6.5^{c}±0.1
Group II	6	33.7^{b}±0.4	37.8^{b}±0.5	37.2^{b}±0.6	9.8^{b}±0.2
Group III	6	22.3^{c}±0.4	28.4^{c}±0.7	32.2^{c}±0.8	14.2^{a}±0.2
F (p)		436.547* (<0.001*)	888.065* (<0.001*)	552.959* (<0.001*)	585.189* (<0.001*)

Data are presented as means±SE. N=Six experimental animals per group.

Mean values with similar letters are insignificant. *Statistically significant at P≤0.05.

Table 3: Effect of maternal betamethasone exposure before fertilization on some biochemical parameters in cerebellum tissue of newborn rabbits.

	No.	Ach E (U/mg protein)	SER (ng/mg protein)	DOP (ng/mg protein)	GABA (ng/mg protein)	Caspase-3 (ng/mg protein)
Group I	6	64.7^{c}±0.5	106.0^{a}±1.0	196.0^{a}±1.0	27.4^{c}±1.5	33.7^{c}±0.6
Group II	6	72.7^{b}±0.7	81.5^{b}±0.5	175.2^{b}±0.9	32.8^{b}±0.4	48.8^{b}±0.6
Group III	6	112.0^{a}±0.9	65.2^{c}±1.0	133.5^{c}±0.8	38.4^{a}±0.7	90.3^{a}±0.6
F (p)		1354.062* (<0.001*)	536.694* (<0.001*)	1201.923* (<0.001*)	30.587* (<0.001*)	2222.075* (<0.001*)

Data are presented as means±SE. N=Six experimental animals per group.
Mean values with similar letters are insignificant. *Statistically significant at P≤0.05

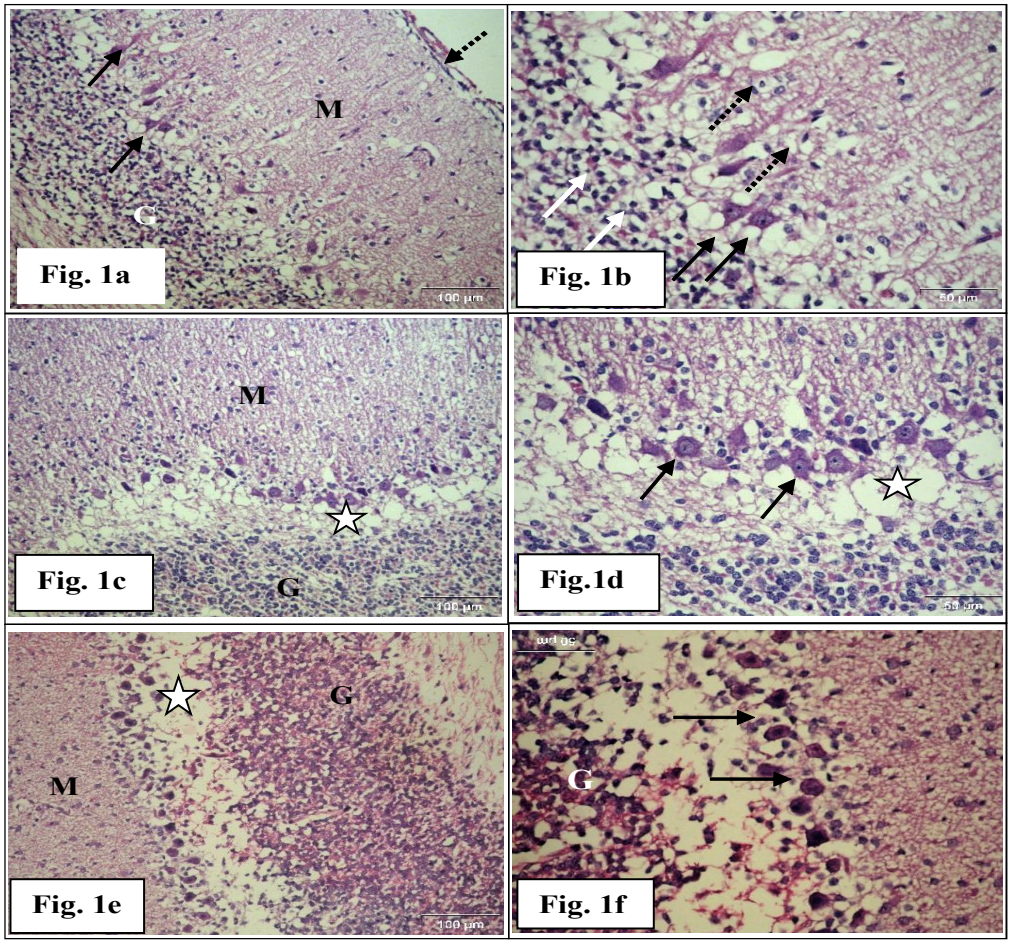

Fig 1: (1a) Light micrograph of control cerebellar section of newborn rabbits stained with H&E showing: the cerebellar cortex tissue containing Purkinje cell layer(arrows), granular (G) and molecular(M) layer. Central core (Co) and meninges (dashed -arrow). Fig (1b): Enlarged part of Fig (1a) showing: small neurons (white-arrows) in granular layer, single row of large neurons with clear extensions (black-arrow) in Purkinje cell layer and primarily glial cells (dashed-arrows) in molecular layer. Fig (1c): Light micrograph of cerebellar cortex tissue of low dose betamethasone -treated newborn rabbit stained with H&E showing: cerebellar cortex tissue with wide separation (star) between Purkinje cell layer and granular layer (G). Note lose molecular layer (M) with microgliosis. Fig (1d): Enlarged part of Fig (3) showing: affected cerebellar cortex have necrotic Purkinje cells with ill-defined arborization (arrows) surrounded by vacuolated area (star). Fig (1e): Light micrograph of cerebellar of high dose betamethasone -treated newborn rabbit stained with H&E showing: the cerebellar cortex tissue with thick granular layer (G) separated from the Purkinje cell layer (star) with more microgliosis in molecular layer (M). Fig (1f): Enlarged part of fig (1e) showing: Purkinje cells with dark-staining nuclei, scanty cytoplasm and degenerated dendrites (arrows). Note, the presence of highly vacuolated area separating granular layer (G) from Purkinje layer by. Figs 1a,1c, 1e, × 100, Figs 1b,1d,1f × 400.

can induce apoptosis in cerebellar tissue of these newborn rabbits resulting in neuronal death. The suggestion may be supported by other workers (Vose *et al.,* 2013). This may be due to the physiological role of glucocorticoids in controlling programmed cell death in the mammalian cerebellum (Noguchi *et al.*, 2008).

At the cellular level, endogenous and exogenous glucocorticoids regulate cell proliferation, differentiation and apoptosis and glucocorticoids are powerful mediators of vascular function (Michael and Papageorghiou, 2008). These cellular effects are critical but antenatal glucocorticoids also act on the developing brain. Exogenous glucocorticoids increase cerebral vascular resistance leading to decreased cerebral blood flow (Miller *et al.*, 2007) and impair cerebral oxygen delivery in a region-specific manner (Schwab *et al.*, 2000). These changes in cerebral blood flow are associated with altered electrocortical activity, suggestive of dysfunctional complex neuronal activity and disturbed cerebral metabolism (Schwab *et al.*, 2001). In particular, they induce acute hyperexcitability and sustained alterations in ovine fetal sleep patterns (Davidson *et al.*, 2011). At the cellular level, synthetic glucocorticoids disrupt myelination within the brain of appropriately grown fetal sheep (Antonow-Schlorke *et al.*, 2009) and reduce the neuronal number in fetal primates (Uno *et al.*, 1990).

Effect of maternal betamethasone exposure before fertilization on histological structure of cerebellum of new born rabbits

In control cerebellar cortex tissues, the Purkinje cell layer appeared in between granular and molecular layers surrounded from outside by meninges (Fig 1a). Densely packed small neurons in granular layer, single row of large neurons with clear extensions in Purkinje layer and primarily glial cells in molecular layer were observed. Neurons in Purkinje layer have single axon extending into the granular layer and multiple dendrites branching in the molecular layer. The axons of the small neurons in granular layer extend into the molecular layer. The axons of granule cells and the dendrites of Purkinje cells are shown in molecular layer (Fig 1b). In cerebellar tissues of G II, there was widespread degenerative changes appeared as spacing and dissociation between Purkinje layer and granular layer with lose molecular layer. Molecular layer showed ore proliferation of glial cells (Fig 1c). The Purkinje cells had cell bodies with marked irregularity in cell boundary, mild eosinophilia with ill-defined arborization into the molecular layer were illustrated (Fig 1d). Cerebellar tissues of GIII demonstrated extremely severe degenerative changes where more spacing and dissociation between Purkinje layer and granular layer were illustrated (Fig 1e). The shrinked Purkinje cells have pyknotic nuclei with no arborization surrounded by cavity were showed. Due to no arborization of the pyramidal cells, molecular layer and granular layer appeared with low content (Fig 1f).

The present study revealed histological alteration in cerebellum cortex of groups II and III. Similar results were obtained by Noguchi (2014) under glucocorticoid treatment. The observed nuclear and cytoplasmic changes in studied tissues in betamethasone treatment groups might be indication of oxidative stress and lipid peroxidation damage of DNA and other cytoplasmic macromolecules which may induce damage in membranes and causes degeneration of cells (Badawy *et al.*, 2016).

CONCLUSION

Accordingly, it was concluded that the treatment of mother with betamethasone, 2 months before fertilization, adversely affect cerebellum of newborn rabbits by traid biochemical and histological changes. So, according to the above mentioned, it is recommended to stop betamethasone treatment by mother before fertilization by enough period, more than 2 months, as it accumulates in the blood of them resulting in serious alterations in the embryos.

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Ethical statement

The experimental protocol of this investigation was approved by Institutional Animal Care and Use Committee (IACUC) at the King Faisal University with Research Ethics Committee number: KFU-REC/2021-03-05.

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Conflict of interest: None.

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