

Effects of Topiramate Administration on Placenta of Rat

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Background: Topiramate was classified as pregnancy category D, which means that it possesses a potential risk to the fetus. An increasing evidence points to the risk of development of cleft lip and/or cleft palate (oral clefts) in infants born due to Topamax (topiramate) during pregnancy.

Objective: To evaluate the effects of the therapeutic doses of topiramate on the placental structure.

Design: An Experimental Animal Study.

Setting: Teratology Laboratory, Anatomy Department, CMMS, Arabian Gulf University.

Method: Pregnant rats were treated with oral topiramate at doses of 50 and 100mg/Kg body weight. On day 20, the pregnant rats were sacrificed, and the placentae were collected and processed for histological evaluation.

Result: Degenerative changes in all three layers of the placenta (decidual, basal and labyrinthine) were observed. In the decidual layer, deposition of fibrous and hyaline materials were found in the cells, in addition to vacuolization and hemorrhages. In the basal layer, the trophoblast cells (giant, basophilic and glycogen cells) showed vacuolization, cytolysis and cyst formation. In the labyrinthine layer, there was an increased fibrinoid material and fetal mesenchyme. In addition, degeneration of cells and congestion of blood vessels were evident.

Conclusion: The deleterious effects of the therapeutic doses of topiramate on the placental structure may play a role in its teratogenicity. These placental changes are not dose dependent.

Bahrain Med Bull 2016; 38 (2): 90 - 93

Topiramate (TPM), an antiepileptic drug, is indicated as initial monotherapy or as an adjunctive therapy in patients with partial onset or primary generalized tonic-clonic seizures¹. Recently, TPM has been evaluated for other neurological or psychiatric disorders such as migraine, bipolar disorder, borderline personality disorder, post-traumatic stress disorder and in combination with phentermine for the management of obesity²⁻⁶.

Topiramate is a pregnancy category-D drug. Exposure of pregnant animals to topiramate, in clinically relevant doses, resulted in structural malformations including skeletal defects and reduced fetal weights in off-springs⁷. Topiramate should be used during pregnancy only if the potential benefits outweigh the potential risks. There is limited information on topiramate-induced changes in placenta. Rats treated with high doses of Topiramate from day 9 to 12 of gestation revealed placental pathological changes⁸.

The placenta is a very important channel for the exchange of materials between the maternal and fetal blood⁹. The placental cells are the major source of the hormones required for the growth and development of the embryo^{10,11}. These hormones ensure pregnancy maintenance and fetal growth and development^{12,13}. The placenta is composed of several

cell types, among which are trophoblast cells. These cells play a primary role in protecting the embryo from noxious xenobiotics, provide maternal support and prevent maternal immune rejection¹³. They also ensure nutrient/waste transfer required for normal growth and maturation of the embryo¹⁴. Rats resemble humans in that they exhibit hemochorial placentation; however, they have a shorter gestation period with a fully functional placenta present for only one week^{13,15,16}. This makes the rat a reasonable model to study some aspects of placental development. Limited data exist in the literature regarding the effects of antiepileptic drugs on the placental structure.

The aim of this study is to evaluate the effects of the therapeutic doses of topiramate on the placental structure.

METHOD

The following is the second part of the method used for our project which was divided into four parts (Ribs and Vertebrae – published, the other two are pending)⁷. Female Sprague-Dawley rats weighing 150–250g were kept at the animal house, Arabian Gulf University - Bahrain, in an environmentally controlled room with ambient temperature (12-hours light/dark cycles) in spacious wire mesh cages. Two weeks later,

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one fertile male rat was placed into each cage with two females overnight. Pregnancy was detected by the presence of spermatozoa in the vaginal smear next morning, and this was considered the first day of gestation. The present study was done in healthy (non-epileptic) pregnant rats⁷. The pregnant rats were then randomly allocated into three groups of eight each.

All groups received food and water *ad libitum*. Topiramate was dissolved in distilled water (10 mg/mL) and was administered by intra-gastric route from day 6 through day 19 of gestation.

Group I, the control group received distilled water, Group II received 50 mg/kg TPM daily and Group III received 100 mg/kg TPM daily. On day 20 of gestation, the rats were sacrificed with inhaled ether overdose. The placentae were obtained and fixed in Bouin's solution. Three placentae were chosen randomly from each mother per litter with a total of twenty-four placentae in each group. The specimens were processed for preparation of paraffin blocks. Paraffin sections (5 μ m) were mounted on glass slides and serial sections were stained with hematoxylin-eosin (H&E). Processing and staining techniques were done according to Bancroft and Gamble¹⁷. Slides were examined and photographs were obtained using a light microscope with the camera.

RESULT

Histological examination and the structural changes of the placentae of rats treated with topiramate are illustrated in figures 1 (a-i).

The changes are seen in the different layers as darkening and pyknosis of nuclei (arrows) and deposition of hyalinized material (H) with degeneration and multiple cyst formation (C). Giant cells (G) with their dark fragmented nuclei and perinuclear halo in some of them and basophilic cells (B) with vacuoles are noticed. In addition, vacuolization and hemorrhagic cysts (star) containing many red blood cells are seen, and glycogen cell (GI) clusters were found. An increased deposition of the fibrinoid material and abundant fetal mesenchyme (M) in some areas compared to scanty fetal mesenchyme in the control is seen.

The decidua of the control group was comprised of small flat, oval cells with bundles of fibers and homogenous cytoplasm, see figure 1(a). They had flat to oval, and relatively dark nuclei occupying most of the cytoplasm. In the decidua layer of TPM50 group, marked degeneration of the cells (vacuolization) was seen, see figure 1(b). Some cells contained more fibrous materials particularly at the periphery of the decidua. Wide areas of congestion and hemorrhagic cysts containing many red blood cells in this layer were found. In the decidua layer of TPM 100 group, there was deposition of acidophilic hyalinized areas and vacuolization of several cells with a decrease in size of their nuclei, see figure 1(c). Congested areas with hemorrhages throughout the layer were observed.

The basal layer of control group consisted mainly of three types of trophoblast cells: giant, basophilic and glycogen cells, see figure 1(d). The giant cells were big oval cells with pale cytoplasm and pale central nuclei. They were located near the decidua layer. They were found sometimes singly or in groups. The basophilic cells (spongiotrophoblasts) had basophilic cytoplasm and central vesicular nuclei. The glycogen cell, mostly arranged in clusters, had very pale cytoplasm with very fine fibers and central nuclei.

In the basal layer of TPM50 group the giant cells were frequent and increased in size, see figure 1(e). Some cells showed signs of degeneration, smaller darker nuclei, deposition of dark acidophilic substance in the periphery of the cells. Other cells showed signs of death, markedly dark cytoplasm and small dark (pyknotic) peripheral nuclei. Some basophilic cells (spongiotrophoblasts) showed vacuoles in their cytoplasm and degenerated pyknotic nuclei. Glycogen cells became more numerous and prominent and occupied most of the thickness of the basal layer. Many of them underwent cytolysis and degeneration and were replaced by empty spaces creating big cysts. There was more deposition of fibrinoid material within the cell clusters.

In the basal layer of TPM100 group, there was degeneration and increase in the apoptotic cells, see figures 1(c) and 1(f). Multiple hemorrhagic cysts appeared which showed cytolysis involving various cell types. The giant cells became more abundant and increased in size. Their cytoplasm was more acidophilic and filled with vacuoles and some of them showed perinuclear halo. Some cells became degenerated and their nuclei were darker and fragmented. Basophilic cells (spongiotrophoblasts) showed degeneration, in the form of vacuoles in their cytoplasm. Their nuclei also appeared degenerated, decreased in size and some of them were pyknotic. Glycogen cells became more numerous and prominent and occupied most of the thickness of the basal layer. There was also increase in number and size of cell clusters. Many of these cells underwent cytolysis and degenerated and were replaced by fibrinoid material.

The labyrinthine layer of the control group consisted of fetal capillaries and maternal sinusoids, see figure 1(g). It was characterized by its longitudinal arrangement in plate-like configuration. Few fetal mesenchymes in the placental barrier between maternal and fetal blood were seen. This layer had the syncytiotrophoblast cells as well as few trophoblast giant cells found scattered through the labyrinthine layer. The labyrinthine layer of TPM50 group showed distortion of their plate-like architecture, see figure 1(h). Deposition of more fibrinoid material and perivascular fibrosis and abundant fetal mesenchyme were found. Fetal blood vessels were mostly hyalinized and showed an increased cellular debris and congestions. The trophoblast giant cells were more frequent and in some areas they were aggregated in groups in the walls of the labyrinth. They appeared degenerated with dark nuclei. The labyrinthine layer of TPM100 group revealed similar changes as TPM50 group, see figure 1(i). Fetal blood vessels showed an increase in cellular debris, vascular congestions and the presence of hemosiderin deposits. Excess glycogen cell clusters in the walls of the labyrinth were found.

DISCUSSION

In this study, the administration of topiramate to pregnant rats resulted in degenerative changes affecting all layers of the placenta: congestion and hemorrhages as well as fibrosis, deposition of hyaline material and vacuolization of cells. The two therapeutic doses of topiramate have been used and the results obtained are variable however they are not dose dependent. Vascular congestion has been reported in the placentae of female rats exposed to lead during pregnancy¹⁸. This is probably associated with enhanced fetal-maternal exchange surface which could be a compensatory mechanism to facilitate the transport of fluids from the vascular system. The vascular changes in TPM-treated placentae may be to neutralize its toxic effects. A similar change was reported in

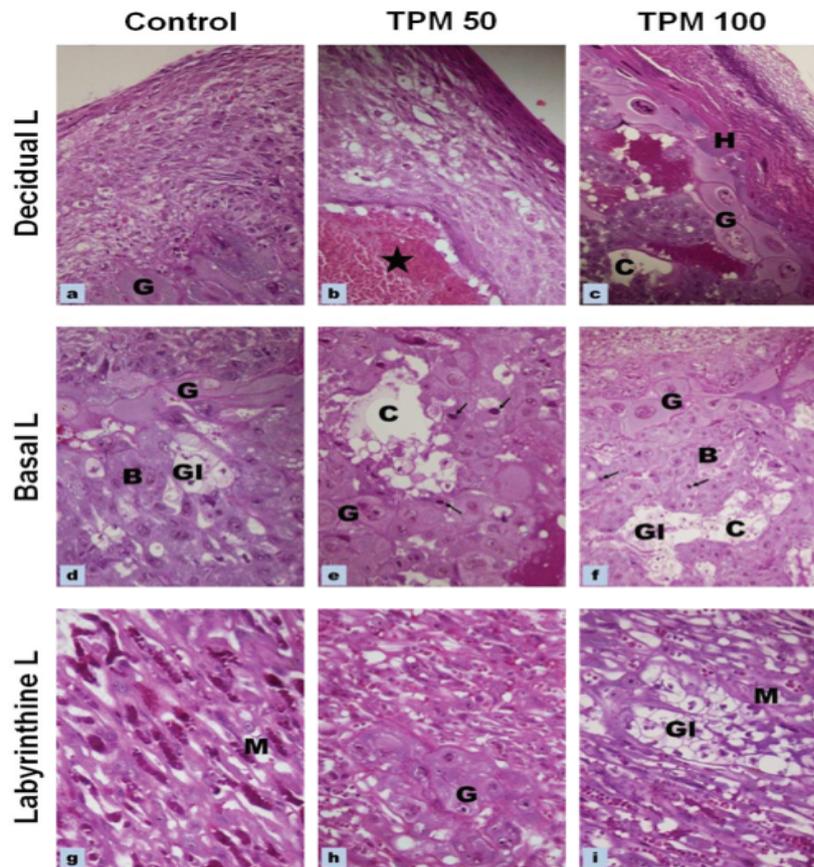


Figure 1: (a-i) Structural Changes in the Different Layers of the Placentae (Decidual, Basal and Labyrinthine) of the Rats. Note the Changes in the Topiramate 50 Mg (b,e,h) and Topiramate 100mg (c,f,i) Treated Groups When Compared to the Control (a,d,g) Group. H&E X400 (Except “f” X200)

methyl parathion exposed rats: hemorrhages in the decidual layer¹⁹. This might indicate a direct toxic effect of TPM on the uterine blood vessels.

The basal zone is the site of production of proteins, hormones and energy products²⁰. The present study showed an increased thickness of the basal layer. This could be due to the increase in the three main cellular components of the basal layer namely giant, basophilic and glycogen cells, in addition to hemorrhages and cysts formation.

The giant cells have phagocytic properties which serve to eliminate dead or degenerated cells^{13,21}. The current study showed an increased number and the size of the giant cells. Also, they were aggregated in groups in the walls of the labyrinthine layer. This may be attributed to the deleterious effects of TPM administration on cells of the maternal-fetal interface. This probably stimulates the giant cells to phagocytose and remove damaged and dead cells which lead to their increase in size and number. Similar to our results, the number of giant cells increased in the basal zone of the placentae of 6-mercaptopurine (6MP) treated rats^{20,22}.

In our study, although there were some degenerative changes in the basophilic cells (spongiotrophoblasts), the increase in their number is considered a derangement of the normal development. Spongiotrophoblasts have some immunological function and are known to decrease in their proliferative activity than other trophoblastic cells with the advance of pregnancy^{13,20}. Moreover, it has been reported that the mechanism of action

and side effects of antiepileptic including topiramate may involve modulation of immunologic reactions²³. The increase in the number of spongiotrophoblast in our study might indicate stimulation of the immune system against the toxicity of Topiramate.

Glycogen cells in rats normally store glycogen and are proposed to function as energy reservoirs^{13,24}. The fetus obtains considerable energy from this glycogen especially during late gestation which is critical for fetal growth. Our results revealed an increase in the frequency and clusters of the glycogen cells associated with some degeneration and fibrous deposition within the clusters and the formation of cysts. Similar changes have been reported in streptozotocin-induced diabetes in rats, which resulted in extensive degenerative changes of glycogen cells and their replacement by cysts²⁴. The presence of glycogen cell-like clusters in the walls of the labyrinthine layer, in the present study, might be attributed to the need of energy against TPM toxicity. On the other hand, a decrease in the glycogen cell population has been reported, in the 6MP treated rat placentae, which might involve a different mechanism since this drug has anti-proliferative effect²⁰.

The current study revealed degenerative changes in the labyrinthine layer, distortion of their longitudinal architecture and deposition of fibrinoid material and perivascular fibrosis and an increase in fetal mesenchyme. The increase in fibrinoid materials and the fetal mesenchyme, which normally disappear in late stages of pregnancy, could be a mechanism by which the placenta is increasing the placental barrier as a mechanical

factor to decrease the placental permeability towards TPM. The persistence of large quantities of fetal mesenchyme that contributes to thick placental barrier seen in diabetes is similar to our results with topiramate²⁴.

In the present study, we found apoptotic cells: pyknosis in the nuclei and an increased phagocytic activity and cell debris. Cell apoptosis in the basal layer and labyrinthine layer was reported with prenatal exposure to 6MP in rats²². Additionally, the presence of trophoblastic cell degeneration and death was similarly observed in methyl parathion exposed rats¹⁹. This might suggest a direct toxic effect of TPM at the cellular level.

The present study revealed an increased vascular congestion as well as an increased cellular debris and the presence of hemosiderin deposits. TPM is reported to have extensive transplacental transfer; this might change the osmolarity of blood vessels which affect the growth and development of the fetus^{25,26}.

CONCLUSION

Topiramate administration resulted in degenerative changes of all layers of rat placentae. We suggest that these changes contribute to topiramate teratogenicity. However, therapeutic doses are not dose-dependent.

Author Contribution: All authors share equal effort contribution towards (1) substantial contribution to conception and design, acquisition, analysis and interpretation of data; (2) drafting the article and revising it critically for important intellectual content; and (3) final approval of manuscript version to be published. Yes.

Potential Conflicts of Interest: None.

Competing Interest: None.

Sponsorship: Arabian Gulf University, Bahrain.

Submission Date: 20 March 2016.

Acceptance Date: 4 April 2016.

Ethical Approval: Approved by the Research and Ethics Committee at the College of Medicine and Medical Sciences, Arabian Gulf University, Kingdom of Bahrain.

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