Topiramate induced histopathological changes in placenta of rats

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Received 13 December 2007; revised 11 August 2008

Effect of topiramate, an antiepileptic drug, on the development of conceptus and its safety during pregnancy has been investigated in experimental rats. Rats were treated with topiramate dissolved in tap water in the doses of 40, 100 and 200 mg/kg body weight from day 9 to 12 of gestation through oral route. Fetuses along with placenta were collected for examination on day 21 of gestation after sacrificing the pregnant rats with deep ether anaesthesia. The placenta showed neither significant reduction in weight as compared to the controls nor any overt anomaly. However, on microscopic examination all treated groups showed similar structural changes which increased in severity with increase in the dose of the drug. The deciduas basalis showed thickening, haemorrhage and increased fibrinoid deposit. Disproportionately increased frequency of vacuolated giant cells was observed in the basal zone whereas frequently broken trichorial membrane, homogenous labyrinthine septa and increased fetal mesenchyme were observed in the placental barrier. The results suggested that topiramate induced dose dependent deleterious changes in the structure of placenta, therefore it should be used with caution during pregnancy.

Keywords: Decidua basalis, Fibrinoid matter, Gestation day, Giant cells, Glycolysis, Mesenchyme

Topiramate (TPM) is a sulfamate substituted derivative of structurally occurring monosaccharide D-fructose i.e. 2-3: 4-5- bis-O- (1-methyl ethylidene)-β- D– fructopyranose sulfamate. TPM is a structurally unique anticonvulsant. It is widely used for treatment of patients with epilepsy irrespective of reproductive age, sex and status of pregnancy mainly due to its effectiveness in maximal electro shock test, its relative long duration of action and its high neuroprotective index.

TPM is known to produce limb agenesis1, cause nephrolithiasis2, induce degeneration of cells in brain3, reduce effectiveness of oral contraceptive pills with low estrogen content4 and also acts as an anorexic agent5. However, the effect of this widely used antiepileptic agent on the placental structure has not been reported so far though the role of placenta in normal development is an established fact and the relation of placental pathology to abnormal embryogenesis has also been well documented6-11.

Effect of clinically equivalent doses of topiramate on placenta in rats as it is a strong link in production of drug induced teratology has been studied. Effects of maternal administration of topiramate on the micromorphology of rat placenta collected on day 21 of gestation are reported in the present study.

Materials and Methods
Adult female albino rats (45) of Charles Foster strain were used in the present study after prior approval from Institutional Animal Ethics Committee. All experimental rats weighing 180±10 g were housed under standard laboratory conditions (25° ± 2°C, 12 hr L/D cycle, 60 % RH) and were fed with Hindustan Lever diet and tap water ad libitum. The rats were kept for mating in the evening, with female to male ratio of 2:1. Presence of sperms in vaginal smear on the following morning indicated pregnancy and was designated as day zero (0) of gestation. The pregnant rats were divided into 2 groups, control (15) and treated (30). The treated group was again divided into 3 groups of 10 each. Each group was given topiramate (Topamac, Cilagag, Johnson & Johnson Lab) dissolved in tap water from day 9 to 12 of pregnancy at 09.00 hrs through oral route by intubation in the doses of 40, 100 and 200 mg/kg body weight respectively. The corresponding control rats (5 with
each drug treated group) were given equivalent volume of tap water through the oral route at 09:00 hrs. All the control and treated pregnant rats were sacrificed on day 21 of gestation by deep ether anaesthesia and fetuses and placentas were collected, examined and weighed individually. The formalin fixed placentas of control and treated groups were sectioned at 8 µ thickness and stained with hematoxylin and eosin stain for morphological observations.

Results

No significant reduction was observed in the weight of fetuses and placentas of topiramate treated groups as compared to those of controls. There was no significant mortality in any of the groups but overt anomalies such as growth retardation, flexion deformity of the limbs, syndactyly, ectrodactyly and brachydactyly were observed. On alizarine red staining, bony deformities such as fusion of coccygeal vertebrae and missing metatarsal and tarsal bones were observed.

Although no gross anomaly was observed in the treated placentas, the histological sections showed marked pathological changes in all the 3 treated groups as compared to the controls (Fig. 1). But when compared within the treated groups, the structural changes were found to be similar in different dose groups but the severity of the changes increased with increase in the dose of the drug. Structural changes observed in the 3 zones of placenta—Zone 1 (decidua basalis), Zone 2 (basal zone), and Zone 3 (labyrinthine zone) are described here-under:

Zone I—Decidua basalis was relatively thickened and hyalinised consisting of homogenous acidophilic mass (Figs 2 and 3) in the treated cases as compared to controls (Fig. 1). Extensive hemorrhages were frequently observed in this area. The fibrinoid deposit was markedly increased and continuous with fibrinoid material in the deeper zones of placenta. (Fig. 4). Structural changes observed in the 3 zones of placenta—Zone 1 (decidua basalis), Zone 2 (basal zone), and Zone 3 (labyrinthine zone) are described here-under:

Zone II—Basal zone of treated placenta showed all 3 types of cells i.e. small giant cells, large giant cells and glycogen containing cells like the control ones but the frequency of large giant cells was disproportionately increased and the cells looked to be enlarged in 40 and 100 mg groups (Figs. 2 and 3) as compared to those in the controls. The giant cells were vacuolated and contained ground glass like nuclei. Many of the glycogen containing cells had undergone cytolysis and were replaced by fibrinoid material. In the group treated with 200 mg, the cytolysis of glycogen containing cells was more, the giant cells looked to be reduced in frequency as well as in size with degenerating nuclei and the intercellular matrix was replaced by fibrinoid material which was more in intensity as compared to the other two treated groups (Fig. 4).

Zone III—Labyrinthine zone of the control placenta showed well defined labyrinths and blood filled maternal sinusoids bordered by trichorial membrane. There was very scanty fetal mesenchyme in the placental barrier between maternal and fetal blood (Fig. 5). In all treated groups the labyrinthine septa of the treated placenta had lost their architecture and often remained homogenous with increased fibrinoid material and fetal mesenchyme in the placental barrier. At many places the trichorial membrane was broken facilitating the mixing of fetal and maternal blood.

The placental barrier was remarkably thicker in many of the treated placenta. The thickness of placental barrier increased with the increase in dose of the drug. This was primarily due to the failure of fetal mesenchyme to disappear and simultaneous increased amount of fibrinoid material. The trabeculae, compared to 40 mg group (Fig. 6), were thicker in 100 mg group (Fig. 7) and still thicker in 200 mg group (Fig. 8) as compared to the 100 mg group. In 200 mg group extensive fibrinoid deposition was observed in the trabeculae. The fetal blood vessels were mostly hyalinised. Increased mass of cellular debris was also observed.

Discussion

There are a few reports about topiramate induced congenital malformations however, placental changes induced by the drug have not been reported. The placenta not only provides a link between the circulation of two distinct individuals (maternal and fetal) but also acts as a barrier to protect the fetus from xenobiotics in the maternal blood. However, the impression that the placenta forms an impenetrable obstacle against most drugs is now widely regarded as false. It has been shown that nearly all drugs that are administered during pregnancy will enter, to some degree, the circulation of fetus via passive diffusion although the extent transfer varies considerably. Parameters considered as possible factors determining the extent of placental transfer are the molecular weight of drug, PKa of drug and extent of drug binding to plasma protein. Still, the extent of drug binding to plasma protein does not influence the type
of drug transfer across the human placenta. The existence of differential systems, including plasma membrane carriers, biotransforming enzymes and export pumps etc. are known to determine the selectivity and efficacy of the so called placental barrier. Topiramate reduces voltage gated Na⁺ current in cerebellar granule cells, enhances postsynaptic GABA receptor current and limits activation of the AMPA Kainate subtypes of glutamate receptors. It lowers neuronal pH due to combined effect on Na⁺ independent Cl⁻/HCO₃⁻ exchange and carbonic anhydrase. The apparent decrease of steady state pH may contribute to anticonvulsive property of TPM. Therefore, it may be suggested that via Na⁺ channels TPM may affect the osmolarity of blood vessels which interacts with various factors responsible at the time of growth and development of fetuses.

Fig.1–4—1: Placenta of control group showing the three layers –Zone I (decidua basalis), Zone II (basal zone), and Zone III (labyrinthine zone). H & E, × 107. 2: Placenta of TPM (40 mg) treated group showing thickened decidua basalis with acidophilic mass (A) and increased fibrinoid deposition (F). H & E, × 168. 3: Placenta of TPM (100 mg) treated group showing thickened decidua Basalis, increased acidophilic material (A) in zone I and fibrinoid deposition (F). H & E, × 268. 4: Placenta of TPM (200 mg) treated group showing increased fibrinoid deposition (F) and haemorrhagic spots (H). H & E, ×168.
Topiramate is also reported to have extensive transplacental transfer\textsuperscript{16}. The present results have demonstrated that topiramate causes definite structural changes in rat placenta. Extensive fibrinoid deposition in labyrinthine and basal zone and haemorrhage in the decidual zone were observed and also the placental barrier was substantially thickened. This is because of failure of fetal mesenchyme to disappear and substantial amount of fibrinoid matter deposition. This pathology may be attributed to osmolarity changes in

Fig. 5–8—5: Labyrinthine zone of control placenta showing placental barrier (↑). H & E, × 672. 6: Labyrinthine zone of TPM (40 mg) treated placenta showing thickened placental barrier (↑) due to deposition of fibrinoid material. H & E, × 409. 7: Labyrinthine zone of TPM (100 mg) treated placenta showing markedly thickened placental barrier, increased fetal mesenchyme and fibrinoid deposition (↓). H & E, × 256. 8: Labyrinthine zone of TPM (200 mg) treated placenta showing extensive fibrinoid deposition (F), hyalinised fetal blood vessels (B) and cellular debris. H & E, × 409.
the blood caused by topiramate\textsuperscript{17}. It has been proved that topiramate inhibits placental carnitine transport resulting into its deficiency in fetuses which has been associated with anticonvulsant syndrome\textsuperscript{18,19}. This inhibition of carnitine transport may be due to increase in fibrinoid deposition and thickening of placental barrier thus giving us a reason for the pathogenesis of teratological changes in fetuses of mother given topiramate during pregnancy.

References