EFFECT OF PRENATAL TOPIRAMATE EXPOSURE ON BEHAVIORAL ALTERATIONS IN RATS

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Abstract

Pregnant Charles-Foster rats were exposed to topiramate, a novel antiepileptic drug at the doses of 40, 100 and 200 mg/kg body weight orally from day 9-12 of gestation (GD). The animals from both treated as well as vehicle control groups were allowed to deliver on GD 21. The offspring culled at birth on the basis of sex and weight were subjected to behavioral tests at the age of 8 weeks. The topiramate treated rat offspring showed enhanced anxiety, increased fearfulness, reduction in spatial learning and memory on behavioral tests performed in Open field, Elevated plus maze and Morris Water Maze. These findings suggest that prenatal exposure to topiramate during a critical period of brain development leaves a lasting imprint on the brain, resulting in abnormal anxiety states, possibly through dopaminergic neurotransmission mechanisms.

Key Words: Seizures, Hippocampus, Anxiety, Learning, Neurotransmission.

Introduction

Topiramate (TPM), a novel antiepileptic agent classified as a sulfamate substituted monosaccharide, was approved by Food and Drug administration in 1996. It is classified chemically as 2,3: 4, 5 bis – O (1 methyl ethylidene) - β - D – fructopyranose sulfamate, its empirical formula being C_{12}H_{21}NO_{7}S (1).

Initially approved for partial seizures in adults, when used in addition to other drugs TPM is effective in both adult and children with refractory partial seizures with or without secondary generalized tonic-clonic seizures. It acts as an anticonvulsant primarily by blocking the spread of seizures rather than by raising the seizure threshold (2). It has been reported to be a promising medication for the treatment of both alcohol and nicotine dependence, presumably by its ability to modulate corticomesolimbic dopamine function profoundly (3).

It has been reported that it produces limb anomalies in rats (4,5), renal calculi in patients (6) and reduces effectiveness of oral contraceptives in human beings (7). Though in a single case only, minor anomalies have been reported in an infant born to a mother treated with topiramate (8), and also abnormal behavioral changes have been observed in patients treated by the drug(9). A single experimental report is available about impaired learning function accompanied with improved motor function in rodents treated by topiramate one month after experimentally induced traumatic brain injury (10).

It has already been established that prenatal exposure to other neuroleptic agents such as Penfluridal and chlorpromazine results in significant reduction of DA from the mesolimbic dopaminergic system and decreased noradrenaline turnover in striatum and hippocampus, thus inducing prolonged behavioral deficits in treated subjects (11,12). Since dopamine rich sites in the limbic areas and basal ganglia are specially vulnerable to centrally acting drugs, this may be responsible for long lasting behavioural dysfunctions in rat offspring (10,13-16).

It still remains to be determined whether neurochemical and neuroanatomical alterations in fetuses are accompanied by long lasting behavioral dysfunctions in rat offspring, so we can only speculate about the nature of the foetal neuromicromorphological changes and altered behavioral responses in adult rat offspring treated prenatally with topiramate. In view of the paucity of reports about topiramate induced behavioral changes, if any, the present study has been planned.

Material and Methods

Animals

Inbred Charles- Foster rats (180 ± 10 g) born and acclimatized in our laboratory were used for the present study. These rats were maintained under standard laboratory conditions, viz. 24 ± 2 ºC room temperature, 60 ± 10% relative humidity and 12 h light (0600 to 1800 h)/12 h dark cycle (1800 – 0600 h). All animals were housed in transparent acrylic cages with rice bran bedding. The pelleted food and tap water were provided ad libitum throughout the experiment. Bedding was changed twice a week.

Determination of pregnancy

The male and nulliparous female rats (1:2) were caged together overnight for mating. On the next day at 08.00 h, mating was inferred by the presence of sperm in vaginal swab and the day of sperm positivity was designated as Gestation Day (GD) 0. The Sperm-positive dams were housed individually in polypropylene cages in the same laboratory conditions.

Experimental design and drug exposure

All sperm positive female rats were randomized into groups, i.e. group 1 (n = 30), group 2 (n = 15), group 3 (n = 15) and group 4 (n = 15).

The topiramate tablets manufactured by CILACAG, Scafhausen, Switzerland and marketed by Johnson and Johnson Ltd. were procured, crushed and then dissolved in tap water.

The group 2 was given 40mg, group 3 was given 100 mg and
group 4 was given 200 mg of topiramate from day 9 to 12 of gestation days through oral route at 09.00h. The group 1 again divided into 3 groups (10 each) was given equivalent volume of tap water from day 9 to 12 of gestation days through oral route at the same corresponding time with the corresponding treated groups. Twenty four hour food intake was recorded daily in all the groups (Control and treated) from GD 0 to 20 and also the dams were weighed daily from GD 0 to 20 at 09.00 h. The pregnant dams from all the groups were allowed to deliver naturally. At birth [postnatal day (PND)1] rat pups from each litter were culled to six pups (4 males, 2 females) per litter on the basis of body weight. The culled pups were reared by their biological mothers up to weaning (at PND 21) after which female pups were discarded from the study. Only male rats from each litter were raised up to PND 56 (at 8 weeks). At this stage, two male rats were selected from each litter, i. e. 9 litters (18 rats) in control, 15 litters (30 rats) in 40 mg , 12 litters (24 rats) in 100 mg and 12 litters(24 rats) in 200 mg groups. At this stage some rats from each litter were also sacrificed for neurohistological study to determine whether topiramate may induce permanent deleterious effects on the brain. Male offspring used in this study were weaned at birth (PND 1) and then once a week, till the age of 8 weeks (PND 56).

Behavior tests

Open Field Exploratory test

An open field apparatus made of plywood measuring 60.96 x 60.96 x 60.96 cm was used to test the open field exploratory behavior of rats. The floor of the apparatus was divided into 16 evenly spaced squares surrounded by opaque high walls of 60.96 cm. The entire apparatus was painted black except for the 6 mm wide white lines that divided the floor into 16 squares. The open field was illuminated by 100 W bulb focusing into the field from a height of about 100 cm from the floor. The entire room except the open field was kept dark during the experiment. In the novel test situation, each animal was centrally placed in the test apparatus for a maximum of 5 minutes to observe the following behaviors.

1. **Ambulation**: The number of squares crossed by the rat
2. **Rearing**: the number of times the rat stood on its hind limbs
3. **Self grooming**: the number of responses of grooming, scratching, licking and washing made by each individual rat
4. **Fecal pellets**: the number of fecal pellets excreted by each individual rats. Before each trial, the floor and the walls were cleaned with cotton soaked in 70% alcohol.

**Elevated plus maze test**

This model was used to test the anxiety pattern in rodents. The plus maze consists of two opposite arms 50 x 10 cm, connected with a central square (10 x 10 cm), giving the apparatus a shape of plus sign. One arm painted white was kept open, whereas other arm was enclosed with 40 cm high walls and was painted black together with the walls. The maze was kept in a dimly lit room and was elevated 50 cm above the floor. The experimental animals were placed individually in the centre of the maze facing an enclosed arm and the time spent on the open and closed arms were recorded during the next 5 minutes for each rat. An arm entry was recorded when all four paws of the rat entered the arm. Like the open field apparatus, the floor and walls of open and enclosed arms were cleaned with 70% alcohol before each trial.

**Morris Water maze test**

The maze consists of a black circular pool (diameter 2.14 m, height 80 cm) filled to a depth of 44 cm with water maintained at 25°C. The water was made opaque by adding a few drops of India ink. On first day, the animals (i.e. rat pups of approximately 8 weeks age) were habituated by exposing them in water maze for 1 minute for spatial learning and memory test. On the second day, a random sequence of four starting poles along the perimeter of the pool was generated which divided the maze into four quadrants. A circular platform (9 cm diameter) was then kept hidden 2 cm below water level in the centre of one of the quadrants. Each rat pup was placed in the water facing wall of the water maze at one of the poles which was considered as the starting location and was allowed 90 seconds to find the hidden platform where it was allowed 20 seconds rest. After that it was lifted out and again the same sequence of testing procedure was started from the second next pole. This was repeated for third and fourth poles also. The time taken by the animal to find the hidden platform was noted in all the four trials.

On third day of the testing the platform was elevated above the water level and the animal was placed in the water facing walls of the water maze at the starting location and was allowed 90 seconds to come to the platform. The time taken was noted for all the animals. This was repeated for all the four poles. Such two sessions of four trials were conducted.

On fourth day, one session of such four trials was conducted after four hour interval of which the platform was removed and a probe trial (without platform) was conducted. Each rat was placed in the pool at the same randomly selected starting pole and the swimming path was observed. The time spent in looking for platform in the quadrant of pole which initially contained platform was measured.

**Statistical analysis**

First, mean ± S.D value for each group for different variables was calculated. Then one way analysis of variance (F test) was used to find out the significance of difference in the mean level of different variables among the groups. If this test resulted as significance of difference then Multiple Range (Dunnett’s test) was applied to find out the pairwise difference between control Vs treated groups.
Results

Maternal Toxicity
There was no significant effect on body weight gain of the drug treated mothers as compared to those of controls.

Changes in body weight of offspring:
No mother died in any of the groups during gestation and lactation. Since there was non significant reduction in the body weight of topiramate treated rat offspring from PND 1-56, the data is not presented here.

Behavioural Teratology
Open field test
Prenatally topiramate treated rat offspring displayed significantly decreased (p<0.001) ambulation in the group treated with 40 mg of topiramate, but it was significantly increased in the group treated with 100 (0.05) and 200 (0.001) mg of the drug. The immobility was significantly increased in the group treated with 40 mg (p<0.001) and significantly decreased in the group treated with 100 and 200 mg ((p<0.001) topiramate. Thus the ambulation was significantly increased and the immobility was significantly decreased in 100 and 200 mg dose groups though there was reverse effect with low dose (40) mg of the drug. Significant decrease (p<0.001) in rearing was observed in all 40, 100 and 200 mg topiramate treated groups. In self grooming there was significant decrease (p<0.05) in the group treated with 40 mg, highly significant (p<0.001) decrease in the group treated with 100 mg and non significant increase in the group treats with 200 mg of the drug. There was non significant decrease in the number of fecal pellets in all the treated groups (Table –I).

Table-I: Effect of prenatal topiramate exposure on open field exploratory behavior in rat offspring.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Open-field exploratory behaviour</th>
<th>Values are Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of fetuses (N)</td>
<td>Ambulation (N)</td>
</tr>
<tr>
<td>Control (Vehicle)</td>
<td>18</td>
<td>53.5±3.21</td>
</tr>
<tr>
<td>Topiramate (mg/kg) 40</td>
<td>30</td>
<td>18.3±2.2***</td>
</tr>
<tr>
<td>100</td>
<td>24</td>
<td>55.8±2.51*</td>
</tr>
<tr>
<td>200</td>
<td>24</td>
<td>76.6±4.68***</td>
</tr>
<tr>
<td>F value</td>
<td>764.98***</td>
<td>4736.24</td>
</tr>
</tbody>
</table>

Dunnetts test --- Comparison between control Vs treated groups * = p< 0.05** = p<0.01*** = p<0.001

Elevated plus Maze test
Results obtained on elevated plus-maze behavior test indicate that prenatally TPM treated rat offspring of all groups (40, 100 and 200mg) spent significantly(p<0.001) less time in open arms and more time (p<0.001) in closed arms as compared to those of control rat offspring. These tests indicate enhanced anxiety and increased fearfulness (Table-II).

Table-II: Effect of prenatal topiramate administration on elevated plus maze behavior in rat offspring.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Fetuses (N)</th>
<th>Responses on elevated plus-maze Values are Mean ± SD Time spent (Sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Open arms</td>
</tr>
<tr>
<td>Control (Vehicle)</td>
<td>18</td>
<td>88.3±10.5</td>
</tr>
<tr>
<td>Topiramate (mg/kg) 40</td>
<td>30</td>
<td>10.5±3.1***</td>
</tr>
<tr>
<td>100</td>
<td>24</td>
<td>39.5±5.23***</td>
</tr>
<tr>
<td>200</td>
<td>24</td>
<td>33.3±4.8***</td>
</tr>
<tr>
<td>F value</td>
<td></td>
<td>637.16</td>
</tr>
</tbody>
</table>

Dunnetts test --- Comparison between control Vs treated groups *** = p<0.001
Morris Water Maze test

The result of Morris water maze test for spatial learning and memory indicates that TPM treated rat offspring of all groups (40, 100, 200 mg) took significantly more time to reach the platform in all sessions as compared to the offspring of controls. This shows comparative reduction in spatial learning and memory induced by the drug even with a low dose of 40mg for 4 days only (Table - III).

Table-III : Effect of prenatal topiramate administration on Morris water maze test behavior in rat offspring.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Fetuses (N)</th>
<th>Time to reach the hidden platform from poles (sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
</tr>
<tr>
<td>Control</td>
<td>18</td>
<td>15.16±3.5</td>
</tr>
<tr>
<td>Treated</td>
<td>40</td>
<td>41.5±5.1***</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>27.6±4.05***</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>23±3.61***</td>
</tr>
<tr>
<td>F value</td>
<td></td>
<td>168.69</td>
</tr>
</tbody>
</table>

Dunnetts test -Comparison between control Vs treated groups*** = p<0.001

Discussion

There was nonsignificant weight reduction in the body weight of treated rat offspring from PND 1 to 56 which may be attributed to the effect of topiramate because there was no considerable drug induced anorexia or maternal catalepsy.

In open field test, the treated rat offspring displayed significantly increased ambulation and decreased immobility in the group treated with 100 and 200 mg of topiramate, but there was significant decrease in ambulation and increase in immobility in the group treated with 40 mg of the drug. This reversal of effect of the drug between low and high doses cannot be extrapolated at this stage. The rearing was significantly decreased in all the treated groups. Self grooming was decreased in the 40 and 100 mg groups but was nonsignificantly increased in the 200 mg treated group. There was nonsignificant decrease in the number of fecal pellets also. In elevated plus maze these offspring made significantly less time in open arms and more time in closed arms indicating enhanced anxiety and increased fearfulness. In Morris Water Maze test, the treated animals took significantly more time to reach the platform in all sessions as compared to the offspring of controls indicating reduction in spatial learning and memory.

All these findings indicate that topiramate treated rat offspring when subjected to stressful or new environments, find it difficult to cope with the environment resulting in slower habituation. Similar findings have already been reported from rat offspring treated with haloperidol (11-13,17-20), barbiturate (14,21,22) and diazepam (17,23). On the same lines, it has been reported that out of 130 analyzed patients undergoing TPM therapy, 46 patients developed affective disorder, 22 aggressive behavior, 16 psychosis, 11 anxiety and 8 personality changes (24).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 CI⁻/HCO₃⁻ exchange and carbonic anhydrase (25). The apparent decrease of steady state pH may contribute to anticonvulsive property of TPM (1). Therefore, it may be possible that via Na⁺ channels it affects the osmolarity of blood vessels, which interacts with various factors responsible at the time of growth and development of affected fetuses.

Behavior is the result of the interaction between genes and the environment and the most important mechanisms by which the environment alters behavior are learning and memory. The functioning of the brain is dependent on its composition and structure, that is, on the molecular environment of the mind since there is evidence that mental function and behavior are also affected by changes in the concentrations in brain of any of a number of other substances that are normally present such as L (+) – glutamic acid, uric acid and γ amino butyric acid. Similarly topiramate may be responsible for inducing behavioral changes by altering the molecular environment of the brain due to its mechanism of action.

Therefore, these behavioral alterations in the form of increased anxiety, fearfulness and delayed learning and memory prove that prenatal topiramate treatment from GD 9-12 alters anxiogenic behavioral patterns in postnatal life even at the age of 8 weeks indicating a long lasting imprint of the drug on behavior.

References