Neuroendocrinology Letters No.6 December Vol.26, 2005
 ISSN 0172–780X **www.nel.edu**

ORIGINAL ARTICLE

Reproductive toxic effects of topamax ingestion in female spague-dawley rats

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Submitted: July 10, 2005 *Accepted: September 20, 2005*

Key words: **topamax; female rats; fertility; reproductive effects; reproductive organs.**

Neuroendocrinol Lett 2005; **26**(6):843–846 **PMID:** 16380695 NEL260605A17

Abstract

PURPOSE: The objectives of this study is to investigate the toxic effects of Topamax (100mg/kg/ Body Wight) on the reproductive system after administration to female Sprague-Dawley rats weighting 250–300 g for two time periods 4 and 12 weeks.

METHODS: twenty adult female rats were divided into two groups and exposed to Topiramate diet at a concentration of 100 mg/kg/body weight for two periods of time. First group containing 10 rats received treatment for 4 weeks and a second group of 10 rats received the same dose of treatment for a period of 12 weeks and compared with twenty non-exposed female rats received vehicle treatment. Female rats were allowed mating with males after 10 days prior to the last administration dose. Animals were autopsied under light anesthesia after mating and several parameters were determined including: number of pregnant rats, body and reproductive organ weight, number of implantation sites, viable fetuses, and resorption sites. Assessment of pregnancies in females was measured and the significance of these results was calculated using student's ''t" and Chi-square tests.

RESULTS: The effect of Topamax exposure on fertility was assessed in terms of pregnant rats number, implantation sites, viable fetuses, and resorption sites. Exposure to Topamax for 4 weeks did not have much effect on fertility. Significant decrease in the relative ovarian weights and embryo weights in rats exposed to Topamax were observed. Exposure to Topamax for a 12 weeks resulted in a reduction in the percentage of pregnancies and in the number of implantation sites when compared with controls in both treatment periods. Rats receiving 12 weeks treatment showed an increase in ovarian weights and a decrease in viable fetuses number. These results indicate that long-term exposure of female rats to Topamax causes adverse effects on the reproductive system and fertility.

CONCLUSION: the results of the current study suggest that ingestion of Topamax by adult female rats causes adverse effects on fertility and reproduction.

Introduction

Approximately 1% of the global population is affected by epilepsy, and 1 million are women of childbearing age [1]. Exposure of childbearing women suffering from epilepsy to a variety of anti-epileptic drugs can put infants at increased risk of congenital abnormalities, including intrauterine growth retardation, neural tube defects and microcephaly. A high incidence of these congenital malformations in infants of mothers taking anticonvulsants drags was observed ranging from 4% to 8% [2]. The most common forms of these congenital abnormalities induced by this treatment can be cleft lip/palate, cardiac defects, as will as uro-genital defects (2). It is believed that the risk of these congenital malformations is a dose dependent. A higher incidence can be reached if a combined anti-epileptic drugs in use [1]. It has been recorded that the first generation of anti-epileptic drugs including phenytoin, phenobarbital, valproic acid, and carbamazepine, was associated with teratogenicity. In fact, phenytoin, phenobarbital, and valproic acid are classified as category D where Carbamazepine is listed as category C. In addition, recent evidence suggested that epilepsy itself may cause some congenital malformations. There are three proposed mechanisms for the teratogenicity of anticonvulsants. The first involves the formation of unstable epoxides, which can cause mutagenic effects. The second mechanism involves production of free radicals that are cytotoxic and the third is folate deficiency in which the risk of neural tube defects is increased [3].

One of the new anti-epileptic drugs with multiple mechanisms of action used recently is Topiramate (TPM, Topamax). It was demonstrated, using randomized controlled trials, that it may have a high efficacy in the management of intractable seizures [4]. In addition, this drug is undergoing development for other central nervous system problems such as neuropathic pain, bipolar disorder, and migraine prophylaxis [4].

It was reported however that administration of Topamax could be associated with several side effects including ataxia, confusion, dizziness, weight loss [5] and acute psychotic symptoms [6,7]. Recently, the overall incidence of these adverse effects, even at low doses, has been reported which include an impaired concentration and memory loss, slowed thinking, and word-finding difficulties [8]. Long-term Topamax administration produces adverse effects on fertility and reproductive system in adult male rats [9].

Currently, there is little evidence available concerning the risks of fetal exposure to this new anti-epileptic drug [10]. Some studies reported a small number of malformations without organ specificity and are not easy to interpret because of many confounding factors. Because of the potential teratogenicity of this drug and according to current guidelines in the treatment of epilepsy in pregnancy, it is recommended that its concentration should be established before conception and that monitoring of its concentration should be continued during each trimester and in the last month of pregnancy [11].

Our aim is to study and evaluate the effect of the anti-epileptic drug Topiramate (TMP, Topamax) on pregnancy outcome and on the fetal intrauterine development using female Sprague Dawly rats treated with one dose 100mg/kg/ Body Weight administrated orally for two different treatment periods**.**

Material and method

Animals. 40 adult female Sprague-Dawley rats weighing 250–300g was used in this Study. Rats were raised in the animal house unit in the Faculty of Medicine at Jordan University of Science and Technology under a controlled temperature of 21 ± 1.0 °C on a 12-hr light/dark cycle. Animals were feed with a regular diet (manufactured by the Faculty of Veterinary Medicine at Jordan University of Science and Technology, Irbid, Jordan, according to standard recipes) and water was provided ad libitum. Female rats were randomly divided into four groups of 10 each.

Administration of Topamax. 20 Rats were exposed to treatment with Topamax through an intra-gastric tube at concentrations of (100 mg/kg/body weight, dissolved in tap water) for two periods of time namely 4 weeks (10 female rats representing group 1) and 12 weeks (10 female rats representing group 2). The two groups of rats were allowed drinking water and normal diet ad libitum, in addition, rats were receiving a dose of 100 mg /kg/body weight/day in form of tablets of Topamax that was dissolved in distilled water through an intra-gastric intubations. The two remaining groups (group 3 and 4) receiving normal diet were considered as a control and were allowed access to normal diet and drinking water ad libitum**.**

Fertility test. Animals were observed daily from the first day of exposure to Topamax for clinical signs of toxicity and their body weights were measured weekly. After the two exposure time, treated and untreated control counterparts rats were divided randomly into groups of two rats each and housed with a sexually mature untreated male of proven fertility for ten days to allow mating. The effect of Topamax ingestion on the occurrence of implantation was estimated in the rats and in their control counterparts after the appropriate time of exposure. During this exposure time, namely 10 days, at least two estrous cycles should have elapsed [12]. The untreated male rats were removed from cages and the treated female rats and their control counterparts were killed by cervical dislocation under light ether anesthesia. Autopsy was performed afterward and the following parameters were recorded: the number of implantation sites, the number of viable fetuses, and the number of resorption sites. Furthermore, maternal body weight, uterus weight, ovary weight in addition to the embryo weights was also recorded.

Statistical analysis. Data was expressed as mean ± and standard deviation (SD). The differences between Topamax treated and controlled groups were analyzed using Student't' test [13].

Table 1a. Effect of 4-week exposure to topamax on fertility of female rats

Treatment	No. of pregnant females	No. of implantation	No. of viable fetuses	Rats with resorptions	Resorptions/ total No. of implantation
Control	9/10	9.33 ± 2.39	8.77 ± 2.72	4/10(40%)	5/84 (5.9%)
Topamax(100)	7/10	8.14 ± 4.14	8.25 ± 0.45	5/7(71.4)	24/57 (42.1%)

Results are expressed as means ± SEM.
* p<0.05 significantly different from the control group (Student's *t* test).
† p<0.05, significantly different from the control group (Fisher exact test). ‡ p<0.001.

Table 1b. Effect of 12-week exposure to topamax on fertility of female rats

Treatment	No. of pregnant females	No. of implantation	No. of viable fetuses	Rats with resorptions	Resorptions/ total No. of implantation
Control	9/10	9.33 ± 2.39	8.77 ± 2.72	4/10(40%)	5/84 (5.9%)
Topamax(100)	5/9 †	6.98 ± 2.81 *	6.83 ± 1.85 *	4/5(80%)	19/35(54%)

Results are expressed as means ± SEM.
* p<0.05 significantly different from the control group (Student's *t* test).
† p<0.05, significantly different from the control group (Fisher exact test). ‡ p<0.001.

Table 2a. Effect of 4 weeks exposure to Topamax on maternal body, organ and embryo weights

Treatment	Final body weight(g)	Ovary weight(g) (mg/ 100g Bwt	Uterus weight(g) (mg/100g Bwt	Embryo weight(g) (mg/100g Bwt
Control	268 ± 18.67	0.37 ± 0.05	0.53 ± 0.01	0.34 ± 0.04
Topamax(100)	257 ± 13.56	0.34 ± 0.01*	0.51 ± 0.03	0.31 ± 0.07 †

a Relative weights. Results are expressed as means ± SEM.
* p<0.05, † p<0.01 significantly different from the control group (Student's *t* test).

Table 2b. Effect of 12 weeks exposure to Topamax on maternal body, organ and embryo weights

Treatment	Final body weight(g)	Ovary weight(g) (mg/ 100g Bwt	Uterus weight(g) (mg/100g Bwt	Embryo weight(g) (mg/100g Bwt
Control	268 ± 18.67	0.37 ± 0.05	0.53 ± 0.01	0.34 ± 0.04
Topamax(100)	244 ± 15.65	0.31 ± 0.03 *	0.48 ± 0.08	0.29 ± 0.32 †

a Relative weights. Results are expressed as means ± SEM.
* p<0.05, † p<0.01 significantly different from the control group (Student's *t* test).

Results

Exposure levels and toxicity of Topamax. None of the animals within the 4-week exposure group (group 1) showed any clinical signs of toxicity. However, for the 12-week exposure group (group 2), one animal out of 10, died due to the exposure to Topamax at concentrations (100 mg/kg body weight respectively.

Effect of Topamax on fertility. Table 1a shows the effect of Topamax ingestion for 4 weeks (group 1) on the fertility of the treated female rats. In what it concerns the number of treated female impregnated by control untreated male rats, a non-significant reduction was observed. In addition, the number of implantation sites in the treated rats observed to be slightly decreased with no significant differences between the control and the Topamax treated rats in this group. The number of viable fetuses was equal in the treated female rats when compared with the controls. The percentage of resorption was elevated where the ratio between the resorption and the total number of implantation was observed to be elevated.

Table 1b indicates the effect of ingestion of Topamax for 12 weeks period (group 2) on the fertility of female treated rats. There were significant decreases in the percentage of pregnant rats in the Topamax treated group (p<0.001) when compared with control counterparts. Furthermore, exposure to Topamax resulted in a decrease in the number of implantation sites as well as the number of viable fetuses and this group in a statistically significant (p<0.05) manner. The percentage of resorption sites in treated rats was more increased in this group where the ratio between the resoption and the number of implantation was greatly increased.

Effect of Topamax on maternal organ weights and embryo weights. Table 2a shows that, ingestion of Topamax for 4 weeks resulted in a non-significat reduction in rats body weight where a statistical decrease in the relative ovary weight (p<0.05) was demonstrated.

A significant decrease in the embryo weights in this group ($p<0.01$) was observed when compared with rats from control counterparts. There were no significant differences in the uterine weights obtained from female treated rats when compared to the controls.

Table 2b shows that the ingestion of Topamax for 12 weeks resulted in a significant reduction in the relative ovarian weight ($p<0.05$) when compared to controls. Animals exposed to Topamax treatment shows also a more significant decrease in the embryo weight ($p<0.001$) when compared to controls. No significant differences were observed in the final body weigh nor in the uterine weight in the treated animals with Topamax when compared to controls however, a slight reduction can be noted.

Discussion

In this study we have investigated the effect of exposure of adult female Sprague-Dawley rats to Topamax at dose concentrations of 100 mg/kg body weight on fertility for two time periods 4-week and 12-week. The animal model used in this study has been used previously in several studies to assess the adverse effects of different compounds on reproduction in laboratory animals [9] In addition, the dose concentrations of Topamax used in the current study was chosen carefully and according to previous studies [9]. The dose of 100 mg /kg of Topamax used in this study was selected because of a previously reported toxicity potentials of higher doses of this compound as well as side effects such as decreased body weight and water consumption and clinical signs of toxicity such as dehydration, lethargy and postural headache [15]. This dose was also selected to obtain broader range of information on the effects of Topamax on behavior parameters and reproduction.

We have shown that the exposure of adult female rats to Topamax for 4 weeks had no significant effect on the rats fertility process. However, there was decrease in the relative ovarian weights and a significant decrease in the embryo weight when compared to non-treated control rats. Concurrently, the treatment exposure with the same dose of Topamax using the same strain of rats for 12 weeks revealed a significant decrease in both the relative ovarian and embryo weights when compared to controls.

The present investigation shows that intra-gastric administration of Topamax promoted a decreased in fertility in female Sprague-Dawley rats. The weight of reproductive organs were markedly decreased as shown in Table 2 a and b. It has been postulated that the reproductive organ weights can be closely regulated by androgen hormones [15,16]. This drug may act on pituitary gland and may lead to a decrease in the main hormones influencing pregnancy. The decrease in weight of reproductive organs further confirmed androgen horomone reduction. Whoever, this decrease in the ovarian weights in treated rats is unexplained and needs to be clarified through both hormonal and histological studies. This will help to elucidate whether the weight decrease in the ovaries observed in this study is due to hyperplasia and/or hypertrophy of the tissue component of this organ.

Any decrease in the weight of reproductive organs is under hormonal control. These results, therefore, suggest that any disturbance of the reproductive endocrine functions, may possibly, go hand in hand with multiple sites of toxicity acting along the hypothalamic-pituitary-ovarian-uterine axis.

The main finding of the current study was the significant reduction in the occurrence of pregnancy in rats exposed to topamax for 12 weeks. This decrease may be due to alteration of the reproductive endocrine functions leading to decreased secretion of progesterone which is needed for endometrial alteration at the time of implantation and is necessary for successful impregnation [16,17]. This may also explain the significant decrease in the number of implantation sites leading to decrease the number of viable fetuses. We are now investigating the effect of Topamax exposure on serum progesterone levels.

In conclusion, the results of the current study suggest that ingestion of Topamax by adult female rats causes adverse effects on fertility and reproduction.

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