Teratogenic effects of gabapentin on neural tube and limb development in mice

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Gabapentin (GBP) (C9H17NO2), with the trade name Neurontin, is a new antiepileptic drug that was introduced by Park Davis Company in 1993 for adjunctive therapy of partial and second generalized seizures. Also, GBP was used as monotherapy in epilepsy, relief of neuropathic pain, prophylaxis of migraine, and for relief of post-dural puncture headache. Furthermore, there is some evidence that GBP has some beneficial effect in people with learning disabilities, that GBP is useful in the treatment of generalized vulvodynia, unprovoked. The wide range of action on the CNS of this newer antiepileptic drug may serve not only for clinical seizure suppression, but also for neuroprotection. Gabapentin is very water-soluble, and the half-life of GBP in humans is 5-7 hours and daily dosages range from 900-2,400 mg in adults. Lack of appreciable metabolism, no drug interaction in the body, rapid glomerular filtration rate, and good tolerance of this drug are reasons for its extensive usage. Furthermore, there is a little information on the teratogenic effects of GBP. Low molecular weight and no binding to the plasma proteins, probably causes this drug to transfer from the placental membrane. Some studies on rodents have shown that oral consumption of this drug (1000-3000 mg/kg per day) during the organogenesis period causes delayed ossification of several bones in the skull, vertebrate, and upper and lower limbs.[1] Another report showed that consumption of GBP during pregnancy can cause hydronephrosis and hydroureter in rat fetuses.[2] Although, Montouris,[3] on the basis of prospective and retrospective data of 51 fetuses, including 3 twin gestations, collected from 39 women with epilepsy and other disorders exposed to GBP during pregnancy showed that GBP exposure during pregnancy did not lead to an increased risk for adverse maternal and fetal events.[3] Of course it was noticed that due to the small number of patients examined in this study, additional data from more pregnancies and outcomes are needed. Clearly, the possible long-term effects on reproductive health and pregnancy outcome requires careful attention when antiepileptic drug therapy is being considered for a patient with childbearing potential. For these findings, this research was carried out to determine the teratogenic effects of GBP on limb and neural tube formation when consumed during implantation and neurulation in mice.

This study was carried out on adult female and mature male BALB/c mice, aged 7-8 weeks old virgin, and weighing 28-30 gram in Birjand University of Medical Sciences, Birjand, Iran during 2005. The males were part of the animal house breeding stock with confirmed mating experience. Dry food pellets and water were provided ad libitum with animal house conditions maintained at 20.1-21.2°C, 65.5-65.8% relative humidity, and a 12 h:12 h photoperiod (lights on 0700 to 1900 hours). Approval for this study was gained from the Birjand University of Medical Sciences Animal Care and Ethics Committee. Two females were caged with a male of the same strain overnight. The presence of vaginal plug the following morning confirmed that mating had taken place and was designated as day 0 of pregnancy (gestation day 0: GD 0). Females that did not mate within 2 estrus cycles were excluded from the study. Thirty pregnant mice were randomly divided into 2 experimental groups (10 mice in each group) receiving 20 mg/kg/day GBP in experimental group I, and 26 mg/kg/day GBP in experimental group II and, and one control group (10 mice) that received normal saline. These doses of GBP were the routine doses used for the treatment of patients with 70 kg average weight. The injections were carried out intraperitoneally from GD 1 to GD 10. Gabapentin powder was obtained from a 100 mg capsule from Pharma Science Inc., from Montreal, Canada. Dilution was carried out by normal saline. On GD 18, the pregnant mice were sacrificed under ether anesthesia and the uterus was opened and the umbilical cord cut close to the fetus, each fetus and placenta was then weighed. Each fetus was assigned a number according to its position in the uterine horn, starting with number one at the ovarian end of the left uterine horn. Fetuses were assessed as either alive or dead and any resorptions noted, live fetuses were then euthanized by hypothermia. All live fetuses were measured and examined externally for malformations or deviations from normal growth. Also, each of the fetuses was weighed by sensitive electronic measurement serrations, GT 210 Ohaus Co., NJ, USA and observed by stereo research Microscope, Olympus SZX, Tokyo, Japan.

Differences in body weight between the control and treatment groups were analyzed using a One-Way ANOVA with Tukey post-test. Resorbed implantation frequency was tested using a χ^2 analysis. A P-value of less than 0.05 was considered significant. The data were analyzed with SPSS (version 11.5).

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Fetal observations. Mean±SD of weight of fetuses were 0.82±0.1 in the control group, 0.78±0.08 in group I, and 0.77±0.06 in group 2. Mean±SD of weight of fetuses in the experimental groups was significantly lower than control group (p=0.001). Also, a significant increase (p=0.01) in resorbed fetuses was observed in the experimental groups as compared with the fetuses in the control group. No malformation was observed in the control group.

Fetal malformations in 20 mg/kg/day GBP experimental group (group I). In this group, out of the 130 fetuses, 8 fetuses (6.15%) had birth defects, 4.6% of birth defects were limb anomalies. Neural tube defects (NTDs) (primarily cystica spina bifida in the thoracic region) were observed in 1.5% of fetuses.

Fetal malformations in 26 mg/kg/day GBP group (group II). In this group, out of the 110 fetuses, 12 (10.9%) of them had birth defects, 8.2% of birth defects were limb anomalies, such as upper and lower limb malrotation and micromelia (**Figure 1**). Neural tube defects were observed in 2.7% of the fetuses. The high incidence of limb defects in fetuses of the 2 experimental groups was significant as compared with the control group. The incidence of NTDs in the fetuses of the 2 experimental groups was not significant as compared with fetuses in the control group.

This investigation showed that consumption of GBP during the implantation and neurulation stages can cause severe limb malformations and NTDs in mice fetuses. In spite of expending data on the usage of GBP, there is little information on its teratogenic effects. The Federal Drug Administration has placed GBP in the C group category.[1] This means that there is no evidence on the teratogenic effect of this drug on humans. Also, Montouris[3] reported that GBP exposure during pregnancy did not lead to an increased risk for adverse maternal and fetal events. However, a little primary research on animals has shown that oral consumption of GBP with 1000-3000 mg/kg per day during the organogenesis period can cause some teratogenic effects such as delayed ossification in some bones of the skull, vertebral column, and upper and lower limbs. Furthermore, this report determined other teratogenic effects of GBP such as hydronephrosis and hydroureter.[2] Based on our research, we can classify the teratogenic effects of GBP into 2 categories: limb malformations and NTDs. In this study, we observed limb malformations such as: limb deformity and dislocation and pronounced shortening of limbs (meromelia) in the both experimental groups (20 and 26 mg/kg/day GBP), although the severity of these defects was higher in group II, which received GBP 26 mg/kg/day. These fetuses also, were smaller and paler than fetuses without anomaly. The above findings showed GBP can induce more serious limb defects than the previous reports mention.[2] The second group of birth defects includes NTDs and the most common form was cystica spinal bifida in the thoracic region. These defects also, were seen in the both experimental groups. The occurrence of NTDs in experimental groups showed that GBP like the other antiepileptic drugs such as valproic acid and carbamazepine can affect neural tube development, or can induce neural tube defects, although the incidence of these anomalies was not significant on comparing with the control group. Our findings on the NTDs in fetuses can be supported with a few case reports such as: birth of a newborn with cyclops and holoprosencephaly whose mother consumed GBP during pregnancy.[1] Furthermore, recent studies have shown that components that affect the gamma-aminobutyric acid (GABA) system can cause NTDs. For instance, research has reported that the teratogenic effect of valproic acid and benzodiazepine can be carried out by increasing the GABA concentration and increasing GABA receptors activity. By considering that GBP has structural similarities with GABA, and GBP is an analogue of GABA, therefore, the presentation of NTDs due to GBP consumption in pregnancy should be considered seriously. However, the exact mechanism of the teratogenic effects of GBP is not clear. However, the possible teratogenic effects of GBP may be due to influences in folic acid metabolism and decrease of folic acid level, changes in concentration of retinoic acid, especially 13 Cis-retinoic acids, alterations in GABA concentration. The other possible mechanism include induction of apoptosis, especially in neural tube cells, and production of the free radicals such as epoxide during metabolism of GBP.[4,5]

Although this study showed that GBP cause limb and NTDs in an animal model, further investigations are needed to determine of exact mechanism and range of GBP teratogenicity.

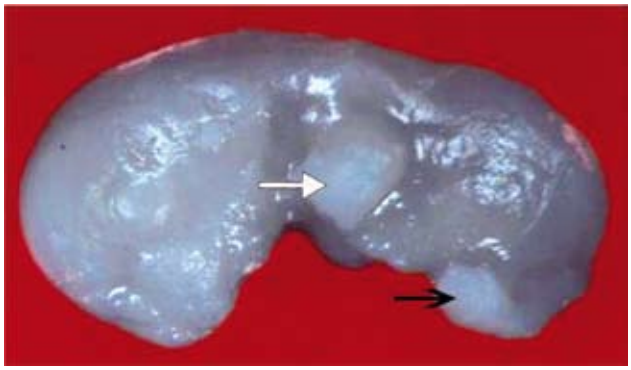

Figure 1 - Fetus with upper (white arrow) and lower limb (black arrow) anomaly from experimental group II receiving 26mg/kg/day gabapentin).

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