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Teratogenicity of Gabapentin in Mice

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ABSTRACT

Introduction: *Present work aims to study and elucidate the safety profile of the antiepileptic doses of gabapentin during pregnancy, and to evaluate if any gabapentin induced murine fetotoxicity at different dose levels.*

Methods: *A total of 60 pregnant mice divided into a total of 12 groups, of 5 mice each were exposed to gabapentin in 4 different doses of 0 (control), or 113, 226, or 452 mg/kg body weight per day, at 3 different gestational stages including early gestation (1-6 day), mid gestation (7-12 day), and late gestation (13-17 day). The pregnant mice were euthanatized on day 18 of gestation, and foetuses were examined for teratogenic manifestations. Brains were dissected out and examined for gross changes, malformations, histological changes, and quantitative protein estimation.*

Results: *Foetal resorptions were observed in all treated groups with gabapentin administration at early gestation (1-6 day), and mid gestation (7-12 day); on the other hand, growth retardation along with stunting in size of live foetuses were observed in all the mid gestation (7-12 day), and late gestation (13-17 day) treated groups. Various gross malformations were observed with all the three doses (113, 226, and 452 mg/kg body weight per day) when with gabapentin was administered at mid gestation (7-12 day). Same trends were confirmed by gross and microscopic examination of brains along with quantitative protein estimation.*

Conclusion: *Gabapentin should not be prescribed in pregnancy, as no therapeutic dose of gabapentin is safe during pregnancy as far as the foetal risk is concerned.*

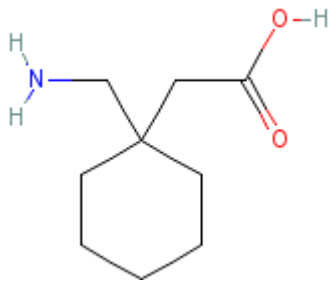
Key Words: *Birth defects, gabapentin, mice, pregnancy, teratogenicity.*

INTRODUCTION

The use of anticonvulsant drugs in pregnancy may have potential effects on embryogenesis, neurological development, growth and subsequent paediatric progress, which presents unique challenges to both the clinicians and their patients [1]. Control of maternal epilepsy including drug selection and dose adjustment, must be balanced with the foetal and neonatal risks associated with anticonvulsant drugs as well as the clinical status of the patient [1]. Teratogenic screening is required and also recommended as drug administration during pregnancy may result in adverse foetal effects. In the general population 1% of the adults and 5% of the children suffers from epilepsy. Which antiepileptic should be prescribed to epileptic pregnant women? Effective, safe and free from foetal toxicity is the choice. No antiepileptic is ideal and safe. Overall, no one drug can be specifically recommended; but monotherapy with most of the recognized first-line drugs have satisfactory outcome. Polytherapy should be avoided if possible as it is associated with greater incidence of congenital malformations and [1]. Gabapentin and lamotrigine have the highest GP awareness rates among the newer anti-epileptics [2]. Therefore there is a possibility that newer second-line agents, like gabapentin, may be safer as add-on therapy [1].

In 1993, Gabapentin was approved by FDA for use in epilepsy. IUPAC Name of gabapentin is 2-[1-(aminomethyl) cyclohexyl]acetic acid. Empirical Formula is $C_9H_{17}NO_2$.

Molecular Mass is 171.237 g/mol.



Structure is shown in figure.

Gabapentin (GBP) the amino acid antiepileptic drug (AED), is indicated for adjunctive use in individuals older than 12 years for the treatment of partial seizures with or without becoming secondarily generalized [3]. Biochemical effects enhancing the ratio of gamma-aminobutyric acid (GABA) to glutamate, ion-channel actions (direct or indirect), and/ or enhancement of nonsynaptic GABA release are the different possible mechanisms of action [3]. The anticonvulsant effect produced is related to the concentration of gabapentin in neurons, by the L-system amino acid transporter that has been involved in its absorption from the gut [3]. Oka et al studied the effect of gabapentin [1-(aminomethyl) cyclohexane acetic acid] on Ca2+ channels which involved the activation of nitric oxide synthase (NOS) in the primary neuronal culture of cerebral cortex in mice [4]. Results suggested that gabapentin inhibits depolarization-induced NOS activation in murine cortical neuronal culture. Blockade of both P/Q-type and L-type Ca2+ channels was associated with NOS activation [4].

Rose and Kam [5] suggested that gabapentin have a unique effect on the voltage-dependent calcium ion channels at the postsynaptic dorsal horns. This may therefore interrupt the series of events leading to the experience of a neuropathic pain

sensation. Gabapentin is considered an important drug in the neuropathic pain syndromes management [5].

Gabapentin is usually considered effective in doses of 900 to 1800 mg daily, in three divided doses, although dose may be increased to 3600 mg in some patients to achieve reasonable seizure control [6]. One-third of women with epilepsy have an increase in seizures during gestational period [7]. Ohman et al. [8] studied the pharmacokinetics of gabapentin (GBP) during delivery, lactation, and in the neonatal period and reported an active transplacental transport of GBP, with accumulation in the foetus as an important consequence [8]. Crawford [7] opined that the two newer anticonvulsants (gabapentin and lamotrigine) appear to be less harmful to the foetus as copmpared to the rest. GBP has been labeled category C on the basis of effects produced on rodent foetuses [3]. Drugs that have been found to be teratogenic in man have resulted in similar effects in experimental animals [9]. Although the second generation antiepileptic drugs are reported to have produced teratogenic effects in various experimental animals; however such data with reference to man is still inconclusive [6].

The developmental toxicity of the anticonvulsant agent gabapentin was studied and evaluated by Petrere and Anderson [10] in mice treated by gavage throughout the period of organogenesis. Mice received different doses of 500, 1000, or 3000 mg/kg on gestation days (GD) 6-15. In mice, both body weights and food consumption were recorded on GD 0, 6, 12, 15, and 18. Each near term (mouse, GD 18) female was euthanatized, necropsies were performed, and litter and foetal data were collected. They reported that no adverse maternal or foetal effects were produced in mice treated with gabapentin in doses up to 3000 mg/kg [10].

Montouris [11] reported a human pregnancy registries data of 51 foetuses, including 3 twin gestations. 39 women with epilepsy and other disorders were exposed to gabapentin during pregnancy. Montouris [11] in his study claimed that the rates of different teratogenic manifestations as miscarriage, low birth weight, and malformation were reduced or similar to those seen in the general population or among women with epilepsy. He in his study concluded that gabapentin exposure during pregnancy is not associated to an increased risk for adverse maternal and foetal events [11].

Following are the objectives and the scopes of the present study

1. To study and elucidate the teratogenic profile of gabapentin in pregnant mice.
2. To evaluate gabapentin induced murine fetotoxicity at different dose levels.
3. To assess the impact of gabapentin induced teratogenicity.
4. Hopefully this study will discern that gabapentin teratogenicity is predictable and/or observed as pronounced specific organ defects.
5. To describe the microscopic changes that occurs after gabapentin administration.
6. To assess changes induced by gabapentin in brain of fetuses by protein estimation.

METHODS

Sexually mature adult Swiss white (ICR) mice with average age of 10 to 12 weeks were mated with the males of the same stock for observing foetal teratogenic effects. Optimum and appropriate humane care was provided to the animals bred and kept in the departmental animal house. A total of 60 pregnant mice divided into a total of 12 groups of 5 mice each was used in the study. Gagapin (gabapentin) was administered in 4 different doses of 0, 113, 226, or 452 mg/kg body weight per day, at 3 different gestational stages including early gestation (1-6 day), mid gestation (7-12 day), late gestation (13-17 day). The day on which sperms were seen in the vaginal smear was taken as day Zero of pregnancy. The pregnant mice were euthanatized with overdose of ether anesthesia on day 18 of gestation, and through abdominal incision uterine horns were exteriorized and inspected for foetal resorptions, whereas live foetuses were collected for further examinations.

0.5 ml per 20 g body weight Normal Saline was used as vehicle and was injected intraperitoneally with drugs. Gabapentin with trade name Gabapin was obtained from INTAS PHARMACEUTICALS LTD, Ahmedabad, Gujarat, India. Gabapentin, in doses of 0, 113, 226, or 452 mg/kg body weight equivalent to 0, 900, 1800, or 3600 mg, respectively of the adult human dose [(doses for mice calculated as described in Ghosh [12]], in 0.5 ml saline per 20 g body weight, was divided in 3 equal doses per day; which were administered at an interval of 8 hours to different treatment groups of mice. Groups receiving 0 mg/kg body weight of gabapentin (vehicle alone) were treated as the controls.

Foetuses were examined for external changes and gross malformations. Then brain was dissected out and examined for gross changes and malformations. Bouin's solution was used as fixative for histological study of brain. They were dehydrated by putting in ascending grades of alcohol and were embedded in paraffin wax. Random histological sections were selected and stained by Haematoxylin and Eosin staining. Brains were serially cut at 10 µm thickness in coronal and transverse plane and were stained with haematoxylin and eosin. A total of 120 foetuses (10 per group and 2 per litter) were selected randomly and 1 section per brain (total 10 x 12 = 120, slides) was examined under microscope. Slides were studied at different magnification for observing histological changes. Both gross photography and photomicrography were performed for study of important and significant changes.Further study for protein estimation was performed in 4 groups treated with gabapentin during mid gestation (7-12 days) which mainly manifest positive teratogenic finding. Two foetuses from each of 20 litters (divided into four groups) were selected randomly and used for the assay mentioned below. Quantitative estimation of proteins: Protein contents in different samples of cell lysates, prepared by repeated freeze thaw were determined by standard Folin's method. 200µL of reagent [alkaline copper solution: 25ml of reagent A (2% Na_2CO_3 in 0.1N NaOH) + 0.5 ml of reagent B

(0.5 % of $CuSO_4.5H_2O$ IN 1% sodium potassium tartarate)] was mixed with 40 µL of cell lysate followed by incubation at room temperature for 10 min. 20µL of Folin-Ciocalteu's Phenol reagent (freshly diluted with water in 1:1 ratio was added to the above reaction mixture and allowed to stand at room temperature for 30 min. Absorbance was measured on an Elisa plate reader (lab: system, Finland) at 620nm with water taken as blank.

Different form of data was tested for statistical significance by using one way analysis of variance (ANOVA) test with the help of statistical software programs (Graph Pad Prism). Any value of $p<0.05$ was regarded as significant.

RESULTS

Gabapentin exposure resulted in foetal resorption in different study groups and were statistically significant in treated groups with drug administration on gestational days [1-6 (6 days)] and [7-12 (6 days)] as compared with the corresponding controls; and the incidence increased with increase in dose from 113 to 452 mg/kg body weight . Incidence of foetal resorption was highest (52.50%), when 452 mg/kg body weight gabapentin was administered during early gestation (1-6 day).

Gabapentin administration resulted in growth retardation along with stunting in size. Crown Rump length of live foetuses were decreased significantly in treated groups with drug administration on gestational days [7-12 (6 days)] and [13-17 (5 days)] as compared with the corresponding controls, and the incidence increased with increase in dose from 113 to 452 mg/kg body weight. Maximum reduction in Crown Rump length (16.8±1.59 mm) as compared to corresponding control (25.9±1.92 mm), was observed when gabapentin was injected in dose of 452 mg/kg body weight on gestational days 7-12 (6 days).

Body weight of live foetuses was reduced in all the treated groups; although they were statistically significant only with drug administration on gestational days [7-12 (6 days)] and [13-17 (5 days)] as compared with the corresponding controls in all three doses given. Maximum reduction in body weight (0.61±0.05 g) as compared to corresponding control (1.51±0.12 g) was observed, when gabapentin was injected in dose of 452 mg/kg body weight on gestational days 7-12 (6 days).

Gross malformations including brachygnathia and pointed snout; open eyes and cataract; thick short neck, rudimentary limbs, and malrotated limbs were observed with all the three doses (113, 226, and 452). They were statistically significant when with gabapentin was administered on gestational days [7-12 (6 days)] as compared with the corresponding controls; whereas the incidence of gross malformations increased with increase in dose from 113 to 452 mg/kg body weight. Brachygnathia and pointed snout were the most common malformation (38.46%) observed when gabapentin 452 mg/kg body weight was administered during gestational day 7-12 (6 days).

Gabapentin exposure resulted in gross changes; as reduction in size, and distortion in shape, of brain collected from the foetuses . Weight of foetal brains decreased significantly in treated groups

with drug administration on gestational days [7-12 (6 days)] and [13-17 (5 days)]; as compared with the corresponding controls. The incidence increased with increase in dose from 113 to 452 mg/kg body weight . Maximum reduction in brain weight (14.86±0.99 mg) as compared to corresponding control (31.47±2.08 mg), was observed when gabapentin was injected in dose of 452 mg/kg body weight on gestational days 7-12 (6 days). Important microscopic changes observed in histological sections from frontal cortex were vacuolization and cavity formation surrounded with irregular arrangement of brain cells, as compared to the corresponding controls; when gabapentin was injected in dose of 452 mg/kg body weight on gestational days 7-12 (6 days).

Effect of gabapentin administration on protein contents of foetal brain: A dose dependent inhibition in the protein content of brain tissue was observed following gabapentin exposure during mid gestation (7-12 days) of pregnancy, as compared to brains of fetuses obtained from control mice ($p<0.05$). The protein contents were found as low as 50% in 452 mg gabapentin treated group.

DISCUSSION

Tomson and Battino [13] in their study reported lack of availability of systematic information on the pharmacokinetics of other newer AEDs (e.g. gabapentin, pregabalin, tiagabine, topiramate or zonisamide) during pregnancy. Johannessen and Tomson [14] suggested that the pharmacokinetic variability is less pronounced and more predictable, for the drugs that are eliminated completely unchanged renally (gabapentin, pregabalin and vigabatrin); on the hand, pharmacokinetic variability of gabapentin is increased which is related to its dose-dependent absorption. Adab [15] in their study concluded that higher doses of gabapentin and newer antiepileptic drugs may increase its teratogenic potential and it is still to be studied if the long term adverse effects are related to intrauterine exposure in the second half of pregnancy.

Petrere and Anderson [10] studied the teratogenic effects of gabapentin in mice with different doses of 500, 1000, or 3000 mg/kg on 6-15 gestation days (GD). They observed no adverse maternal or foetal effects in mice when gabapentin was administered in doses up to 3000 mg/kg. On the contrary, in our study gabapentin in daily doses of 113, 226, or 452 mg/kg body weight (equivalent to 0, 900, 1800, or 3600 mg respectively of adult human dose) on three separate gestational periods resulted in different teratogenic manifestations. Foetal resorption were observed in all treated groups with drug administration on gestational days [1-6 (6 days)] and [7-12 (6 days)]; on the other hand, growth retardation along with stunting in size of live foetuses were observed in all the treated groups with drug administration on gestational days [7-12 (6 days)] and [13-17 (5 days)]. Gross malformations including brachygnathia, pointed snout, open eyes, cataract, thick short neck, rudimentary limbs, malrotated limbs were observed with all the three doses (113, 226, and 452); when with gabapentin was administered on gestational days [7-12 (6 days)]. All the aforementioned teratogenic manifestations

were highest in incidence when gabapentin was administered in daily doses of 452 mg/kg body weight.

Results of gabapentin exposure during pregnancy in experimental animals on foetal brain, including histological observations along with quantitative protein estimation were not reported by any study group earlier.

They is a continuous debate going on regarding the safety of newer anti-epileptic medications AEM, such as lamotrigine (LTG), gabapentin (GBP), tiagabine (TGB) or levetiracetam (LEV). This is added by insufficient data concerning use of specific AEM combinations and their resultant teratogenicity, which further provides scope for ongoing definitive commentary on this issue [16]. AEM-specific national and international birth registries, report different dose related concerns of VPA, CBZ and LTG [16]. Montouris [11] reported a human pregnancy registries data of 51 foetuses, including 3 twin gestations. 39 women with epilepsy and other disorders were exposed to gabapentin during pregnancy. Montouris [11] in his study claimed that the rates of different teratogenic manifestations as miscarriage, low birth weight, and malformation were reduced or similar to those seen in the general population or among women with epilepsy. He in his study concluded that gabapentin exposure during pregnancy is not associated to an increased risk for adverse maternal and foetal events [11]. On the contrary, our study on experimental animals (mice) report wide spectrum of teratogenic manifestations induced by gabapentin.

Human pregnancy registries studies are observational study, thus limiting their relevance. On the other hand, the results related to animal studies, including our present study have the difficulties of extrapolating the results to human disease. Hence, before recommending gabapentin during pregnancy in humans, reports of both animal experimental studies and human pregnancy registries studies should be considered honestly; which must be balanced with maternal and fetal risks along with the personal priorities of patients. In general, the multiple drug therapy is considered more dangerous for the foetus than the mono drug therapy [16, 17]. Gabapentin (GBP) when used in addition to other antiseizure agents is indicated for adjunctive use in the treatment of partial seizures with or without secondary generalization, [3, 6]. Hence, keeping in mind the aforementioned context, gabapentin should not be prescribed; as no therapeutic dose of gabapentin is safe during pregnancy as far as the foetal risk is concerned.

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