Original Article

Teratogenic Effects of Carbamazepine in Mice

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Abstract

Objectives: The aim of this study was to determine the teratogenic effects of carbamazepine (CBZ) in BALB/c mice. **Materials and Methods:** Mature female and male BALB/c mice (25–30 g) were used for all experiments. After standardization of administration and dose of CBZ, animals in the CBZ-treated groups (CBZ 450 mg/kg and 600 mg/kg) were fed on medicinal diet. The dams in the control group were mated on the same day as that of the CBZ-treated dams. After cesarean section (CS), fetal viability status and weights were recorded. Gross histopathological examination of fetuses was conducted to identify alterations in morphology and external or internal organs due to *in utero* exposure of CBZ. **Results:** Out of the nine female animals (three treated on CBZ 450 mg/kg, three treated on CBZ 600 mg/kg and three controls), seven were pregnant, and two (one each from the two CBZ-treated groups) were nonpregnant. All fetuses of the control group ($n = 31$) and CBZ 450 mg/kg treated group ($n = 24$) were live, but eight out of the twenty fetuses (40%) of CBZ 600 mg/kg treated group were dead at CS. The birth weight of the fetuses antenatally exposed to CBZ was drastically reduced (0.71 ± 0.06) when compared to control fetuses (1.67 ± 0.12) ($P < 0.0001$). All the fetuses of the CBZ-treated groups showed stunted physical development. **Conclusion:** Although oral administration of CBZ to mice is a convenient model to study the effect of CBZ to pregnancy, higher oral dose was associated with increased fetal loss. Some of the fetuses exposed to CBZ demonstrated structural abnormalities and low body weight.

Keywords: Antenatal exposure, carbamazepine, mouse, teratogenicity

Introduction

Many antiepileptic drugs (AEDs) have therapeutic applications in epilepsy as well as other neurological conditions such as neuropathic pain, migraine headaches, and psychiatric disorders. Most women with epilepsy will require AED therapy throughout their entire pregnancy to reduce the risk of harmful seizures.

AEDs are associated with an enhanced risk of birth defects. Carbamazepine (CBZ) is one of the older and common AEDs used worldwide. There are many inconclusive studies about the safety of CBZ use during pregnancy.

While some earlier studies tended to clear CBZ as a teratogen, subsequent investigations have tended to label it as a potent teratogen.[1,2] Heart defects are the dominant type of malformations seen in children exposed to CBZ and an increased risk of neural tube defects (NTDs) of 0.5%–1% have also been reported on CBZ exposure.[3] Results from Kerala Registry of Epilepsy and Pregnancy indicate that 6.3% of infants born to women in South Indian population maintained on CBZ monotherapy had cardiac malformations;[4] other studies have reported only a frequency of 0.7%,[5] similar to what is expected in the general population.[6] There are also published data suggesting that exposure to CBZ, could be responsible for fetal malformations but the evidence is not yet clear till date.[7] There is also statistically significant evidence that CBZ if used as the sole antiepileptic agent, causes a doubled risk of fetal malformation.[8]

There have been previous studies which have proven teratogenic effects of CBZ in mice models. There are studies explaining teratogenic, and embryotoxic effects of CBZ are associated with different doses.[9] A study by Sucheston *et al*. showed that CBZ in high doses leads to intrauterine growth retardation which results in low body weight and

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length reduction in mice fetuses.[10] Afshar *et al*. showed that there was weight reduction of mice on the 1st day of delivery regardless of the dose of CBZ.[11] Eye malformations like mild to severe exophthalmos and skeletal deformities such as vertebral and calvarial deformities, brachydactyly and short tail were detected in mice fetuses. In a published article, studying the teratogenic effects albino mice, a significant decrease of body weight, individual organ weight, upper and lower limb length of mice and congenital anomalies such as spina bifida, anencephaly, and oligodactyly were noted in offspring of mice treated with CBZ. Growth retardation and neurodevelopmental toxicity are shown to be evident in mice fetuses depending on the dose of CBZ.[12]

The aim of this study was to determine the teratogenic effects of CBZ in BALB/c mice by the mode of oral administration of CBZ.

Materials and Methods

Animals

BALB/c mouse strain was used in this study. The Institutional Animal Ethics Committee clearance of Sree Chitra Tirunal Institute for Medical Sciences and Technology was obtained to carry out the animal experiments. The animals were housed in individually ventilated cages (IVCs), and care and management were done as per Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines. Mature female and male BALB/c mice (25–30 g) were used for all biological experiments. IVCs contained aspen wood chip bedding material and were maintained at temperature of 22°C ± 2°C and 30%–70% humidity and feed and water was provided *ad libitum* throughout the study. CBZ was dosed in the feed on which the animals were fed and the feed was administered as pellets once a day. The drug-treated groups were fed on medicinal diet according to the respective dosage, starting 2 weeks before mating and continuing to the day preceding the caesarean section (CS). The dams in the control group were mated on the same day as that of the drug-treated dams and subjected to CS.

Standardization of mode of carbamazepine administration

In the initial experiment, two female mice and one male mouse were housed together in IVC and this day was recorded as gestational day GD0. The mice were administered 300 mg/kg CBZ. Water was provided *ad libitum*. The female mice were maintained for 4–5 days with the male mouse after which the male mouse was separated. Pregnancy was confirmed by the increase in body weight of the female mice, and the mice were separated into individual cages. On GD20, both the mice delivered the pups. Mouse 1 delivered eight live pups and mouse 2 delivered ten pups, but seven were killed by the dam itself after delivery leaving behind only three live pups. All the pups from both the mice were examined morphologically and photographs captured to look for the presence of any malformations. Weight of all pups was recorded. None of the pups were reported to have any malformations. This initial experiment was helpful in the standardization of the preparation and administration of medicinal feed (feed with CBZ).

Standardization of dose of carbamazepine

Although a standard dose of 300 mg/kg of CBZ was administered to the mice, none of the offspring showed the presence of malformations or anomalies. The next experiment was conducted by administering 600 mg/kg CBZ to three female mice (mouse 3, 4, and 5) which were housed in a single cage. Medicinal feed was administered to the mice 2 weeks before a male mouse was introduced into the cage for mating. The male was cohabited with the female mice for 8 days and then separated. On the 19th day of the gestational period, mouse 3 and mouse 4 delivered three and one pup, respectively. Mouse 5 showed signs of pseudopregnancy. The size of the litter of both mice that delivered was drastically reduced from that of the previous experiment and could be interpreted as an effect of the high dose of 600 mg/kg CBZ that had been administered. However, the pups of both the litters did not show the presence of any malformations on morphological examination. This experiment led us to standardize the highest dose of CBZ to be administered for this teratology study.

Gross histopathological screening

Since the previous experiments did not reveal the presence of any malformations in any of the offspring exposed to the low or high dose of CBZ, we decided to have CS done on the pregnant dams and collect their fetuses which would be subjected to detailed histopathological screening. In this experiment, we selected three female BALB/c mice each into three groups: first group received CBZ at 450 mg/kg dose, second group received 600 mg/kg dose, and third group was taken as the control group.

After euthanizing the dams by cervical dislocation and subjecting them to CS on GD19, external and internal organs were inspected. From each female mouse, the ovaries, uterus, and placentas were removed and examined. Pregnancy status and number of live or dead fetuses and individual fetal weights were recorded.

A visual external examination of fetal pups was conducted at CS to identify any gross defects in structure due to in-utero exposure of tested drug. Each fetus was also examined under dissection microscope for investigating the external morphology and for any external/internal defects or alterations.

Statistical analysis

The differences between the control groups and the treated groups were reported as mean ± standard deviation. Unpaired *t*-test was used to evaluate birth parameters among the two groups. Differences were considered statistically significant at $P < 0.05$.

Results

450 mg/kg carbamazepine-treated group

Three mice in this group were numbered mouse 6, 7, and 8.

Mouse 6 was nonpregnant with hyperemic uterine horns. The reproductive system mainly the ovaries and uterus were apparently normal. No external and internal lesions detected.

Mouse 7 was pregnant with a gravid uterus. The reproductive system mainly the ovaries, uterus, and placentas were apparently normal. No external and internal lesions detected. Fourteen live fetuses were identified in the gravid uterus [Figure 1].

Visual external fetal examination revealed no malformations, anomalies, and variations but all the fetuses showed stunted physical development when compared to the control group. Shortened lower jaw and hyper flexed neck was noted in one fetus [Figure 2]. Two fetuses were further processed for soft tissue examination.

Mouse 8 was pregnant with a gravid uterus. The reproductive system mainly the ovaries, uterus, and placentas were apparently normal. No external and internal lesions detected. Ten live fetuses were identified in the gravid uterus [Figure 3].

Visual external fetal examination revealed no malformations, anomalies, and variations but all the fetuses showed stunted physical development when compared to the control group. Three fetuses were further processed for soft tissue examination.

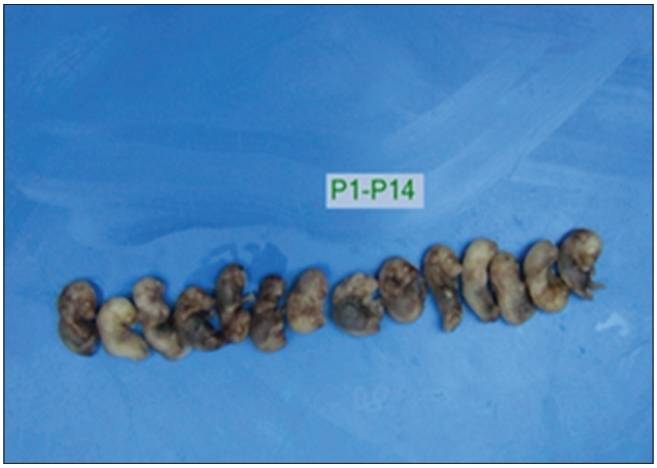

Figure 1: Fetuses of 450 mg/kg carbamazepine-treated group showing stunted growth 14 fetuses of mouse 7

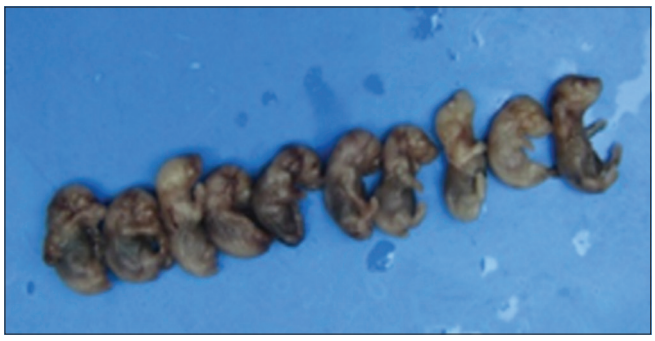

Figure 3: Ten fetuses of mouse 8

600 mg/kg carbamazepine-treated group

Three mice in this group were numbered mouse 9, 10, and 11.

Mouse 9 was pregnant with a gravid uterus. The reproductive system mainly the ovaries, uterus, and placenta were apparently normal. No external and internal lesions detected. Twelve live fetuses were identified in the gravid uterus [Figure 4]. Visual external fetal examination revealed no malformations, anomalies, and variations. Congested cerebral vessels were noted in two fetuses [Figure 5] and in other two fetuses ring hemorrhage at tail base was observed [Figure 6]. Three fetuses were further processed for soft tissue examination.

Mouse 10 was nonpregnant with hyperemic uterine horns. The reproductive system mainly the ovaries and uterus were apparently normal. No external and internal lesions detected.

Mouse 11 was pregnant with a gravid uterus. The reproductive system mainly the ovaries, uterus, and placentas were apparently normal. No external and internal lesions detected. Eight dead fetuses were identified in the gravid uterus [Figure 7]. Visual external fetal examination revealed no malformations, anomalies, and variations but all the fetuses showed stunted physical development. Five fetuses were further processed for soft tissue examination.

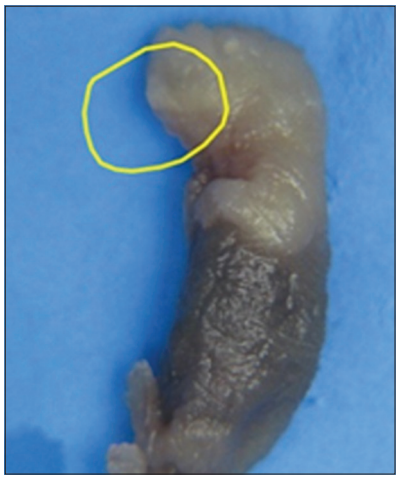

Figure 2: The circled portion of the fetus shows the shortened jaw and hyperflexed neck of one of the fetuses exposed to 450 mg/kg carbamazepine

Figure 4: Fetuses of 600 mg/kg carbamazepine-treated group showing stunted growth 12 fetuses of mouse 9

Control group

The mice selected in this group were numbered as mouse 12, 13 and 14.

Mouse 12 was pregnant with a gravid uterus. The reproductive system mainly the ovaries, uterus, and placentas were apparently normal. No external and internal lesions detected. Eight live fetuses were identified in the gravid uterus [Figure 8]. Visual external fetal examination revealed no malformations, anomalies, and variations. All the fetuses showed normal physical development. One fetus was further processed for soft tissue examination.

Mouse 13 was pregnant with a gravid uterus. The reproductive system mainly the ovaries, uterus, and placentas were apparently normal. No external and internal lesions detected. Fourteen live fetuses were identified in the gravid uterus [Figure 9]. Visual external fetal examination revealed no malformations, anomalies, and variations. All the fetuses showed normal physical development.

Mouse 14 delivered normally just before the elective CS. The reproductive system mainly the ovaries, uterus, and placentas were apparently normal. No external and internal lesions detected. Nine live fetuses were identified in the gravid uterus [Figure 10]. Visual external fetal examination revealed

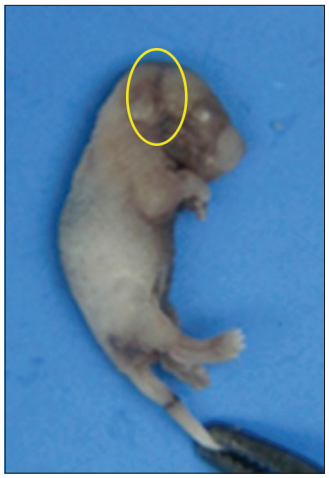

Figure 5: The circle shows congested cerebral ventricles of one of the fetuses exposed to 600 mg/kg carbamazepine

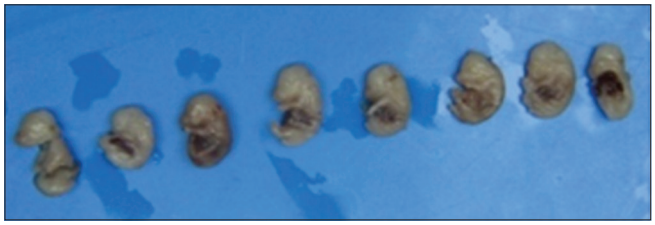

Figure 7: Eight fetuses of mouse 11

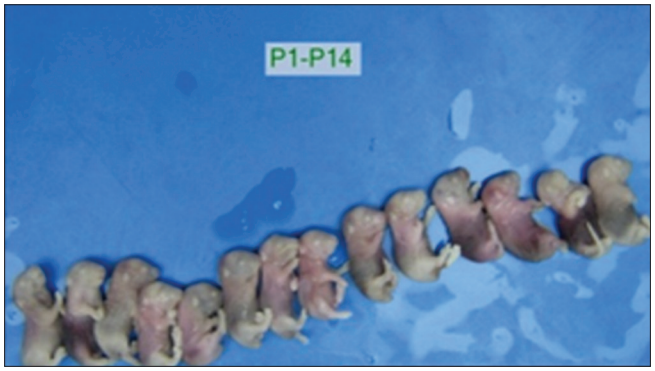

Figure 9: Fetuses of control mouse no. 13

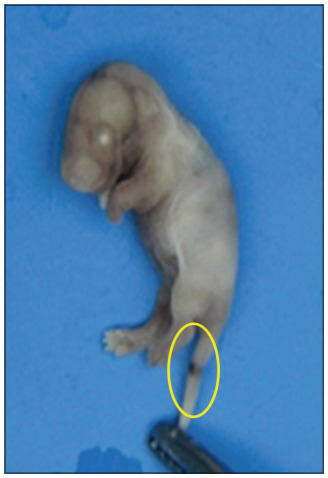

Figure 6: Circle shows ring hemorrhage at tail base

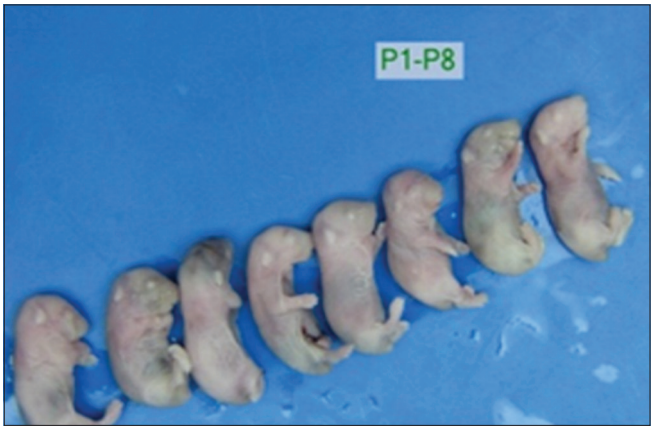

Figure 8: Fetuses of control mouse no. 12

Figure 10: Fetuses of control mouse no. 14

no malformations, anomalies, and variations. All the fetuses showed normal physical development.

There was a notable reduction in the birth weight of the drug exposed fetuses when compared to the control fetuses. Figure 11 clearly shows the stunted appearance of the fetuses exposed to CBZ (T) whereas the control fetuses showed normal development (C).

Table 1 shows the comparison of birth parameters between the control group and the test group. The mean fetal body weights of both experimental groups, 450 mg/kg CBZ (0.71 ± 0.06 g) and 600 mg/kg CBZ (0.72 ± 0.30 g) was significantly reduced compared with those of the control group (1.67 ± 0.12 g) ($P < 0.0001$ vs. control).

Discussion

CBZ is one of the most commonly used AEDs, also used to treat psychiatric disorders and neuropathic pains.[13,14] CBZ rather than CBZ-10, 11-epoxide is found to be embryotoxic to mice embryos when cultured in different concentrations.[15] There are many studies to conclude that CBZ is a teratogen.[16,17] Children of WWE who were on CBZ treatment during pregnancy have increased rates of congenital malformations particularly NTDs, cardiovascular and urinary tract anomalies, and cleft palate and cognitive decline. In various studies conducted previously, decreases in fetal body weight and increases in resorptions and malformations were seen in CBZ-treated mice compared with control groups.[2,18,19] Afshar *et al*., also showed that intraperitoneal administration of CBZ at clinical doses induced several open eye malformations in mice.[11]

Figure 11: Marked difference in the body weight and stunted appearance of the fetuses of the drug-treated group (T) in comparison to the control fetuses (C)

A number of studies demonstrated a growth-restricting effect of CBZ,[16,20] but others found no effect from CBZ on fetal growth.[21,22]

We had selected high CBZ dosages of 300 mg/kg and 600 mg/kg to be administered in mice since our main objective was to elicit the development of malformations in the mice fetuses rather than studying the safe aspects of the drug doses in the animals. In humans, the maximum CBZ dose administered will be between 15 and 20 mg/kg. We had to choose very high doses for our experiments which could be tolerable for the mice dams as well as cause malformations in their fetuses. Our results show that oral administration of CBZ to female BALB/c mice had a remarkable effect on the birth weight of the fetuses compared to that of the control group. The birth weight of the fetuses antenatally exposed to CBZ was drastically reduced when compared to control fetuses. In addition, these fetuses were not alive when they were delivered by CS. However, the gross histopathological and soft tissue sectioning of the fetuses did not reveal any obvious malformations. Probably, this was due to the fact that the CBZ administered orally to the mice could have reached the fetus only in low amounts. A major limitation of our study was that we could not estimate the serum level of CBZ in the female mice, which could have been a factor which explains this result.

This study has helped us to standardize the mode of administration and dose of CBZ which can be tolerated by mice and also proves that CBZ causes low birth weight in fetuses antenatally exposed to the drug. This result can be useful to help clinicians to explain the reduced birth weight of infants whose mothers were on CBZ treatment during pregnancy.

We have to carry out further studies by employing different methods of CBZ administration like the intraperitoneal or intracutaneous mode which would increase the bioavailability in the mice blood and thus pass to the fetus in considerable amounts enough to cause congenital malformations.

Table 1: Birth parameters on gestational day 19 after caesarean section

Parameters	Control group	Test group (mg/kg of CBZ)	
		450	600
Number of litters	3	2	2
Number of fetuses examined (mean±SD)	31 (10.3±3.21)	24 (12±2.82)	20 (10±2.82)
Live fetuses (%)	31 (100)	24 (100)	12 (60)
Dead fetuses	0	0	8 (40)
Fetal body weight (g) (mean±SD)	1.67±0.12	0.71±0.06*	0.72±0.30**

*P<0.0001, **P<0.0001. CBZ=Carbamazepine, SD=Standard deviation

Conclusion

Although oral administration of CBZ to mice is a convenient model to study the effect of CBZ to pregnancy, higher oral dose was associated with increased fetal loss. Some of the fetuses exposed to CBZ demonstrated structural abnormalities and low body weight.

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Conflicts of interest

There are no conflicts of interest.

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