Abstract

Background: Carbamazepine is an antiepileptic drug used widely for the treatment of epileptic seizures and neuropathic pain. Several malformations in humans, mainly neural tube defects, have been reported as a consequence of its use during pregnancy. The association between maternal use of carbamazepine and congenital eye malformations is not very well understood.

Objective: The purpose of this study was to examine this association after intraperitoneal injection of carbamazepine during the period of organogenesis in mice.

Methods: Balb/c timed-pregnant mice were divided into 4 experimental and control groups. Two experimental groups received daily intraperitoneal injections of 15 mg/kg (group I) or 30 mg/kg (group II) of carbamazepine on gestational days 6 to 15. Two control groups received normal saline or Tween 20 (polysorbate 20). Dams underwent Cesarean section on gestational day 18 and embryos were harvested. External examination for eye malformations, routine histological processing of malformed fetuses to study eye morphology, and skeletal staining were performed.

Results: The mean weight and crown-rump of the fetuses in both experimental groups were significantly reduced compared with those of the control groups. Various malformations were detected such as brachygnathia, calvarial deformity, vertebral deformity, short tail, and brachydactyly. Premature opening of one or both eyes with mild to severe exophthalmos occurred in the 2 experimental groups. Deformed lens, retinal folds with undeveloped layers, and corneal folds with absence of surface epithelium were detected in both experimental groups.

Conclusions: This study, to the best of our knowledge, showed for the first time that intraperitoneal administration of carbamazepine at clinically comparable doses during organogenesis can induce several eye malformations in mice. The implication of these results needs to be considered when carbamazepine is administered during human pregnancy.

Keywords: Carbamazepine; ocular malformations; mice; teratogenicity; pregnancy

Introduction

Carbamazepine (CBZ) is an anticonvulsant and mood-stabilizing drug used primarily in the treatment of epilepsy and bipolar disorder, as well as trigeminal neuralgia. It is also used off label for a variety of indications, including attention-deficit hyperactivity disorder (ADHD), schizophrenia, phantom limb syndrome, paroxysmal extreme pain disorder, and posttraumatic stress disorder. It is administered...
mainly by the oral route at doses between 200 and 1,600 mg/day. It is highly effective for partial-onset seizures, including cryptogenic and symptomatic partial seizures. It also has demonstrated good efficacy in the treatment of generalized tonic-clonic seizures, trigeminal neuralgia, neuropathic pain, and mood disorders. This drug is highly effective, well tolerated, and, compared with other antiepileptic drugs (AEDs), considered relatively safe during pregnancy; therefore, it is used regularly by pregnant women. Chemically, CBZ is a neutral, lipid-soluble compound that can easily pass the blood–brain barrier and other membranes in the body [1,2]. In recent comparative clinical trials, it has been demonstrated that CBZ is equally efficacious with, but is less toxic than, phenobarbital, phenytoin, primidone, or valproic acid [3].

Antiepileptic treatment during pregnancy is associated with a 2- to 3-fold increase in the rate of major congenital anomalies, mainly congenital heart defects, cleft lip, and cleft palate; anomalies of the urinary tract; and syndromes of dysmorphism and developmental disability [2]. There is some controversy concerning the teratogenic effects of CBZ, but most investigators believe that malformations associated with maternal use of CBZ can be commonly divided into major malformations such as craniofacial defects, heart defects, and neural tube defects and minor anomalies such as growth retardation, developmental delay, and hyperplasia of the nails or distal phalanges [2,4].

The association between maternal use of CBZ and congenital eye malformations is a subject that has emerged for the field of teratology in the last several years [5]. Sutcliffe et al. have reported 4 unrelated cases of congenital eye malformations in newborns whose mothers were taking CBZ monotherapy during at least the first 2 months of pregnancy [6]. However, some investigators did not find any association between these congenital eye malformations and prenatal CBZ exposure [7].

The objective of this investigation was to show the spectrum of malformations of CBZ at clinically comparable doses administered intraperitoneally (IP) during organogenesis in Balb/c mice.

**Materials and methods**

**Animals, drugs, and treatment**

Virgin female Balb/c mice, weighing 28–30 grams (8–9 weeks old) were used in this study. The animals were maintained in a climate-controlled room under a 12-hour alternating light/dark cycle (0900–2100 hours in light), 20.1°C to 21.2°C temperature, and 50% to 55.5% relative humidity. Dry food pellets and water were provided ad libitum. After 2 weeks of acclimation to the diet and the environment, 3 females were caged overnight with a male of the same strain. The presence of a vaginal plug the following morning confirmed that mating had taken place and was designated as gestational day (GD) 0. Maternal weights were measured daily throughout the experiment. Dams were observed for any signs of clinical toxicity. Forty pregnant mice were randomly divided into 2 experimental groups (10 mice in each group) receiving 15 mg/kg/day of CBZ in experimental group I and 30 mg/kg/day of CBZ in experimental group II, and 2 control groups (10 mice in each group) receiving normal saline (negative control) or Tween 20 (polysorbate 20; vehicle control). The selected doses of CBZ were comparable to the human therapeutic doses in the clinic (15 mg/kg). Dams received CBZ or vehicles daily from GDs 6 to 15 by IP injection. CBZ powder was obtained from Mehr Darou Pharmaceutical Company in Tehran, Iran. Tween 20 (Merck, Darmstadt, Germany) was used as a vehicle to help solubility. Approval for this study was gained from the Animal Care and Ethics Committee of Birjand University of Medical Sciences.

**Fetal assessment**

On GD 18, the pregnant mice were sacrificed under ether anesthesia, the uterus was opened, the umbilical cord was cut close to the fetus, the uterus was removed and uterine weight was recorded, and the corpora lutea was counted and recorded. Each fetus and placenta were then weighed. Fetuses were assessed as either alive or dead and any resorption was noted; live fetuses were then euthanized by hypothermia. The crown-rump of each fetus was measured and each fetus was examined externally for malformations or deviations from normal growth.

**Skeleton and tissue staining**

Malformed fetuses were selected for double-staining of the skeleton. Fetuses were fixed for 3 days in 95% ethanol and then double-stained with alizarin red and alcian blue [8,9] with some modifications, and examined for skeletal anomalies. For tissue staining, fetuses with eye malformations were selected. The heads were removed and fixed in 10% neutral formalin solution. After fixation, routine processes of tissue preparation such as dehydration, clearing, and infiltration were performed and specimens
were embedded in paraffin. The paraffin blocks were trimmed and thin serial sections (3–5 micrometers) were cut with a Zeiss rotary microtome (Zeiss, Jena, Germany). Some sections were randomly selected and stained with hematoxylin and eosin.

Statistics

The unit of analysis was the embryo, and the differences between the control groups and the treated groups were reported as mean ± standard deviation (SD). Analysis of variance (ANOVA) and Tukey’s test were used to evaluate corpora lutea, implantations, and fetal body weight. Resorbed embryos and external malformations were analyzed using $\chi^2$ test when the frequency of each category was 5 or more for resorption or external malformations, and with Fisher’s direct probability test for other cases. Differences were considered significant at $p < 0.05$.

Results

Maternal observations, body weight, and food consumption

All females survived to scheduled study termination on GD 18. There were no postmortem findings judged to be treatment-related. There were no significant differences in absolute maternal body weight in any of the treatment groups at any time during the study. No significant changes in maternal body weight gain were observed in any treatment group during the overall gestational period (GDs 0–18). There were no significant changes in mean food consumption between the experimental groups and control groups (data not shown).

Reproductive parameters

Findings upon cesarean section on GD 18 are shown in Table 1. The mean numbers of corpora lutea and implants were comparable among all groups. CBZ exposure resulted in statistically significant increase in late resorptions in both treatment groups compared with the control groups as analyzed in the compared litters (Table 1). The mean fetal body weights of both experimental groups I (0.72 ± 0.20 g) and II (0.68 ± 0.21 g) were significantly reduced compared with those of the vehicle and negative control groups (1.14 ± 0.19 g and 1.22 ± 0.18 g, respectively). The crown-rump lengths of live fetuses were also reduced significantly in both experimental groups as compared with both control groups (Table 1).

The presence of external malformations such as brachygnathia (mandibular hypoplasia), open eyelid, calvarial deformity (such as frontal bone defect), vertebral deformity (such as scoliosis), short tail, and brachydactyly was considered related to CBZ administration (Table 2). A few fetuses showed severe growth retardation (Figure 1A).

Fetuses with ocular anomalies

Premature opening of one or both eyelids with mild to severe exophthalmos occurred in 8.6% and 7.5% of the fetuses in experimental groups I and II, respectively. Malformations were often bilateral and approximately symmetrical (Figure 1B). Histological findings showed that exposed corneal epithelium was folded and often attenuated. The lens was small and deformed and the retina had a folded pattern with undifferentiated layers (Figure 1C). Fetuses that presented eye, calvarial, and vertebral malformations were also smaller.

<table>
<thead>
<tr>
<th>Table 1. Cesarean section parameters on gestational day 18 in Balb/c mice treated with CBZ.</th>
<th>Control groups</th>
<th>Experimental groups (CBZ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
<td>Tween 20</td>
<td>NS</td>
</tr>
<tr>
<td>Litters</td>
<td>No.</td>
<td>10</td>
</tr>
<tr>
<td>Corpora lutea</td>
<td>No. fetuses examined</td>
<td>148</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>14.8 ± 1.5</td>
<td>15.0 ± 2.0</td>
</tr>
<tr>
<td>Implants</td>
<td>No. fetuses examined</td>
<td>141</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>14.1 ± 1.6</td>
<td>14.3 ± 1.8</td>
</tr>
<tr>
<td>Live fetuses</td>
<td>No. (%)</td>
<td>140 (99.3)</td>
</tr>
<tr>
<td>Resorptions</td>
<td>No. (%)</td>
<td>1 (0.7)</td>
</tr>
<tr>
<td>Fetal body weight (g)</td>
<td>Mean ± SD</td>
<td>1.14 ± 0.19</td>
</tr>
<tr>
<td>Crown-rump length of live fetuses(mm)</td>
<td>Mean ± SD</td>
<td>22.9 ± 1.9</td>
</tr>
</tbody>
</table>

*p < 0.05 vs. control.

*p < 0.01 vs. control.

CBZ = carbamazepine; NS = normal saline; SD = standard deviation.
Skeletal staining of these malformed fetuses revealed some skeletal anomalies such as short, kinked tail and hypoplastic mandible (Figure 1D), scoliosis, wavy ribs, supernumerary ribs, and clinodactyly.

**Discussion**

Women who have used CBZ during pregnancy, especially during the first trimester, have a higher risk of neural tube defects (0.6%–1.7%) [10]. Furthermore, it has been reported that CBZ induces a pattern of minor anomalies and a delay in development in offspring [11] and may result in a “carbamazepine syndrome” characterized by facial dysmorphic features and mild developmental disability [12]. In this study, we focused on the fetuses that had eye malformations, most notably open eye malformations, as it was the most prevalent eye malformation.

In our study, decreases in fetal body weight and crown-rump length and increases in resorptions and malformations were seen in both CBZ-treated groups compared with the control groups; similar findings were reported in other studies [2,13–16]. External and skeletal malformations, including opened eyes, brachygnathia, vertebral and calvarial deformities, brachydactyly, and short tail, were seen in both treated groups (doses of 15 and 30 mg/kg). Furthermore, a few fetuses (4%–6%) in both treated groups had a malformation that could be described as severe growth retardation.

The association between CBZ use by the mother during pregnancy and congenital eye malformations in the newborn in experimental studies is a relatively new subject in teratology. Although some clinical case reports have outlined this association, other experimental studies did not find any cases that showed this relation. Sutcliffe et al.    

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**Table 2.** External malformations in Balb/c mice treated with CBZ.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control groups</th>
<th>Experimental groups (CBZ)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tween 20</td>
<td>NS</td>
</tr>
<tr>
<td>Litters</td>
<td>No.</td>
<td>10</td>
</tr>
<tr>
<td>Fetuses examined</td>
<td>No.</td>
<td>140</td>
</tr>
<tr>
<td>Open eyes</td>
<td>No. (%) litters</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>No. (%) fetuses</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Brachygnathia</td>
<td>No. (%) litters</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>No. (%) fetuses</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Vertebral deformity</td>
<td>No. (%) litters</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>No. (%) fetuses</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Calvarial deformity</td>
<td>No. (%) litters</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>No. (%) fetuses</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Brachydactyly</td>
<td>No. (%) litters</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>No. (%) fetuses</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Severe malformation</td>
<td>No. (%) litters</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>No. (%) fetuses</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Short tail</td>
<td>No. (%) litters</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>No. (%) fetuses</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

*a* No. litters = number of litters having fetuses with the finding (percentage of total litters).

*b* No. fetuses = number of fetuses with the finding (percentage of total fetuses).

*p* < 0.05 vs. control.
have reported 4 unrelated cases of congenital eye malformations: bilateral anophthalmia (1 case), bilateral microphthalmia (2), and unilateral optic nerve coloboma (1). These occurred in the offspring of 4 different mothers who were taking CBZ monotherapy at doses between 200 and 600 mg/day during at least the first 2 months of pregnancy [6]. Two of these children had low birth weight and height at birth. Furthermore, these children showed reduction in corneal diameter and microspherical lens. In the current study, we also found similar findings in mouse fetuses that had open eye malformations. In an investigation of the visual and ocular outcomes of 43 children exposed to prenatal AEDs, the most common of which was CBZ, 2 children presented with severe microphthalmia and one with unilateral optic disk coloboma [5]. In another study, bilateral anophthalmia due to prenatal CBZ exposure was reported [17].

Conversely, some studies have not shown clear evidence of an association between CBZ use during pregnancy and anophthalmia, microphthalmia, or coloboma of the iris or optic disk. Between 1981 and 1999, a total of 288,094 births (live births and stillbirths) took place in the northern Netherlands, and 7,348 fetuses or children with at least 1 congenital anomaly were registered. Among these children, 77 cases with 1 or more eye anomalies were recorded. These included 57 cases of anophthalmia or microphthalmia, 27 cases of coloboma of the iris, 6 cases of optic disk coloboma, and 3 cases of lens coloboma. CBZ use was not reported in any of these cases [7]. Among 8,005 cases of malformation registered with first-trimester drug exposure in the International Database on Malformations and Drug Exposure (MADRE), 46 cases were exposed to CBZ, but no association with any eye malformation was identified [18].

Based on our knowledge, our study is the first experimental study that has indicated an association between CBZ use and eye malformations. On the other hand, compared to our study, in most other animal studies higher oral doses of CBZ were used. For example, CBZ (200–600 mg/kg) in rats increased resorption and skeletal and visceral malformations only at 600 mg/kg. However, its most marked effect at 200 mg/kg and 400 mg/kg was fetal body weight reduction [19]. Nevertheless, all the observed effects occurred at maternal serum concentrations substantially higher, up to 3 times those that are therapeutic for humans. To our knowledge, our study is the first to show that CBZ at clinically comparable doses administered during organogenesis caused embryonic malformations. This possibility could be explained by the route of administration (IP) that we chose to investigate. We believe this needs further investigation to help us better understand the mechanism of this observation.

It is noteworthy that CBZ is metabolized through the arene oxide pathway by which xenobiotics can be hydroxylated through epoxide intermediates [20]. By covalent binding to macromolecules, epoxides may have mutagenic or teratogenic properties. The teratogenicity of CBZ epoxide has been demonstrated in a mouse model [21].

Furthermore, using an embryonic stem cell differentiation system, analysis of tissue-specific gene markers showed that CBZ induced early endodermal and mesodermal differentiation but inhibited differentiation of later developmental stages. CBZ also induced ectodermal development, and there was evidence of neural differentiation, as ES cells with an immature neuronal phenotype were observed [22]. Also, Steinhoff et al. [23] recommended the retina as a parallel model to study the mechanistic properties of anticonvulsant therapy on cortical and cerebral function, because the retina is ontogenetically part of the brain.

Our results suggest further investigation to reveal the spectrum of eye malformations and mechanisms of this finding.

Acknowledgement

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Declaration of interest

The authors have no conflicts of interest in this study.

References

Eye malformations and carbamazepine


