TERATOLOGY 54:45–51 (1996)

In Vitro Embryotoxicity of Carbamazepine and Carbamazepine-10,11-Epoxide

DEBORAH K. HANSEN, STACEY L. DIAL, KETTI K. TERRY, AND THOMAS F. GRAFTON
Division of Reproductive and Developmental Toxicology, National Center for Toxicological Research, Food and Drug Administration, Department of Health and Human Services, Jefferson, Arkansas 72079-9502

ABSTRACT Carbamazepine (Tegretol®, CBZ) is an anticonvulsant drug that is very effective in the treatment of tonic–clonic seizures and is gaining acceptance as a treatment for various psychiatric disorders. The drug is embryotoxic in rodents and has been reported to produce neural tube defects in approximately 1% of prenatally exposed human offspring. It is metabolized by the cytochrome P-450 system to a stable, pharmacologically active epoxide intermediate, carbamazepine-10,11-epoxide. It is currently unknown whether the parent compound, the epoxide intermediate or some other metabolite is the embryotoxic agent. The present study was designed to determine the embryotoxicity of CBZ and its epoxide intermediate (CBZ-E) in a rodent whole embryo culture system. Rat embryos were cultured beginning on day 9 of gestation (GD 9), and mouse embryos were cultured beginning on GD 8. All embryos were cultured for 48 hr in medium containing various concentrations of either CBZ or CBZ-E. Mice were more sensitive to the effects of CBZ than were rats. The parent compound was embryotoxic to mouse embryos at concentrations as low as 12 μg/ml, but it was only embryotoxic at 60 μg/ml to rat embryos. CBZ-E was not embryotoxic to either species at concentrations as high as 48 μg/ml. These results suggest that the parent compound is the embryotoxic agent and that the epoxide intermediate plays no role in the drug's embryotoxic mechanism. © 1996 Wiley-Liss, Inc.*

Carbamazepine (CBZ) is a tricyclic compound that has been useful in the treatment of trigeminal neuralgia and tonic-clonic seizures associated with idiopathic generalized epilepsy (Sillanpää, '93). Recently it has also been found useful in the treatment of psychiatric disorders, including mania, severe depression, and anxiety (Sillanpää, '93). The human therapeutic range is 3–14 μg/ml (Wilder, '92).

Various epidemiological studies have suggested that the malformation rate is higher among epileptic women treated with anticonvulsants than that among nonepileptic women or epileptics not treated with anticonvulsant medications (reviewed by Dansky and Finnell, '91; Schardein, '93). Polytherapy appears to be associated with higher risk of malformation than is monotherapy (Nakane et al., '80; Lindhout et al., '84; Källén, '86; Kaneko et al., '88).

Initially CBZ was believed to be devoid of teratogenic activity (Niebyl et al., '79; Nakane et al., '80). However, in 1989, it was reported that children prenatally exposed to CBZ, alone or in combination with other anticonvulsants, demonstrated a pattern of malformations similar to that produced by phenytoin (Jones et al., '89). These offspring displayed craniofacial dysmorphology (upslanting palpebral fissures, epicanthal folds, and a short nose with a long philtrum), fingernail hypoplasia, and developmental delay. Microcephaly also occurred in a number of these infants and had been reported earlier (Hiilesmaa et al., '81). The conclusion that developmental delay was produced by CBZ exposure prenatally was challenged by Scialli and Lione ('89) and by a later prospective study that demonstrated no differences in global IQ compared to concurrent controls (Scolnik et al., '94).

Although it is unclear whether prenatal CBZ exposure produces a pattern of malformations, several studies suggested an association between neural tube defects (NTDs) and CBZ treatment. In the report by Jones et al. ('89), one infant in a total of 48 had a lumbosacral meningomyelocele. This incidence is similar to that reported by Rosa ('91) in a retrospective study of Medicaid data from Michigan. Lumbar myelomeningocele was also observed in a child exposed to CBZ monotherapy in a prospective study (Gladstone et al., '92). This infant was one of 15 exposed to CBZ monotherapy; eight other children were exposed to CBZ in combination with other anticonvulsant(s). A thoracolumbar neural tube defect was reported in an infant exposed to a megadose of CBZ ingested as a suicide attempt at approximately 3–4 weeks of gestation (Little et al., '93). A recent case-control study found an excess of in-

Received February 2, 1996; accepted April 4, 1996.

Address reprint requests to D.K. Hansen, Division of Reproductive and Developmental Toxicology, National Center for Toxicological Research, 3900 NCTR Rd, HFT-130, Jefferson, AR 72079-9502.

fants with spina bifida after CBZ exposure during pregnancy, but this difference was not statistically significant (Källén, '94). Overall, these studies suggest a risk of about 1% when CBZ is taken during pregnancy.

There are several reports that CBZ is embryotoxic in mice. Enlarged cerebral ventricles were observed among CD-1 mice exposed on GD 6–16 (Sullivan and McElhatton, '77). Dietary administration to CD-1 mice on GD 8–13 produced a decrease in fetal weight (Paulson et al., '79). An increased incidence of malformations was reported in this study with meningocele and spina bifida observed, although no values for the incidence of these defects were reported. Decreased fetal weight and increased orofacial clefting were observed in CD-1 mice treated with several doses of CBZ at various times during gestation (Eluma et al., '84).

Vorhees et al. ('90) reported a dose-dependent decrease in fetal weight and increases in resorptions as well as visceral and skeletal malformations in rats treated with CBZ on GD 7–18. Most of the visceral defects reported were cases of fetal edema; single fetuses with ventricular septal defect, gastroschisis, hydronephrosis, and omphalocele were also noted. Missing ribs and kinked tails were the skeletal defects noted. With the exception of decreased fetal weight at the lowest CBZ dose (200 mg/kg) administered, all embryotoxic manifestations were observed only in the presence of maternal toxicity (as demonstrated by decreased maternal weight gain during gestation).

The mechanism of action for CBZ in developmental toxicity is not understood, but it is known to be metabolized to a pharmacologically active epoxide (CBZ-10,11-epoxide; CBZ-E) as a result of cytochrome P-450-dependent monoxygenase activity. This epoxide is stable and has been quantitated in human urine (Lertratanangkoon and Horning, '82; Eichelbaum et al., '85), plasma (Tomson et al., '94) and cord blood (Pienimäki et al., '95). It appears to be a major metabolite in humans and is nearly completely metabolized to the *trans*-dihydrodiol (CBZ-diol; *trans*-10,11-dihydoxy-10,11-dihydrocarbamazepine) which is excreted in the urine (Eichelbaum et al., '85).

Since the epoxide metabolite is stable and can cross the placenta (Pienimäki et al., '95), there is potential for embryonic exposure. Evidence has been presented to suggest that the epoxide might be partly responsible for the embryotoxic effects of the drug (Lindhout et al., '84). Because of the widespread use of CBZ, the present experiments were designed to determine if CBZ or CBZ-E was able to perturb normal embryonic development in a rodent whole-embryo culture system.

MATERIALS AND METHODS

Animals and animal care

Rats of the NCTR:SDN strain (National Center for Toxicological Research; Jefferson, AR) or mice of the CD-1 strain (originally purchased from Charles River, Wilmington, MA, and maintained at the National Center for Toxicological Research; Jefferson, AR) were used throughout this study. The rats are an outbred albino rat stock originating from Crl:CD® (SD)BR rats obtained in 1979 and maintained as a closed colony. Animal care and procedures followed those in the U.S. Department of Health and Human Services guide (NIH, '85).

All animals were housed in polycarbonate cages with hardwood chip bedding in rooms in which the temperature (20° ± 2°C) and humidity (50 ± 10%) were controlled. Female rats were mated with males overnight, and the next morning was counted as gestational day (GD) 0 if a vaginal sperm plug was found. Lights were on in the rat room from 0700 to 1900 hr daily. Female mice were mated with males for 2.5 hr from 0830 to 1100 hr; if a sperm plug was found, that day was counted as GD 0. Lights were on in the mouse room from 1100 to 2300 hr daily.

Whole embryo culture

Rat and mouse embryos were cultured using the system of New ('78) as modified by Grafton et al., ('87). On the afternoon of GD 9, pregnant rats were euthanized by overinhalation of diethyl ether, and the gravid uteri were removed and placed in sterile Tyrode's solution (Sigma Chemical Co., St. Louis, MO) at room temperature. Under a dissecting microscope, maternal decidual tissue was removed leaving the visceral yolk sac intact. Presomite rat embryos at the early neural plate stage (Fujinaga and Baden, '92) were cultured two per flask at 37°C. Pregnant mice were euthanized on the afternoon of GD 8 by overinhalation of ether, and gravid uteri were removed. Embryos having 2–5 somite pairs were cultured two per flask. All embryos were randomly assigned to culture flasks. All flasks were pregassed for 5 min with 5% O_2–5% CO_2–90% N_2 and regassed for 3 min with the same gas mixture at 1600 hr that afternoon. At 0800 and 1600 hr of the second day of culture, each flask received a 3-min gassing of 15% O_2–5% CO_2–80% N_2. On day 3 of culture at 0800 hr, each flask received a 3-min gassing with 95% O_2–5% CO_2.

Culture medium consisted of heat-inactivated, immediately centrifuged serum from adult female rats. The total volume in each flask was 2.0 ml and was made up of 75% rat serum–25% Tyrode's solution. A penicillin–streptomycin solution (Sigma) was added to each flask (final concentrations: 100 units penicillin, 0.1 mg streptomycin). CBZ or CBZ-E was added to the culture flasks at various final concentrations. CBZ is relatively insoluble in most solvents but could be dissolved directly in serum. This serum was then diluted with untreated serum to achieve the appropriate final concentrations of CBZ.

CBZ-E was synthesized by the Bionetics Corporation under contract to NCTR. The purity and identity of the compound was checked by nuclear magnetic resonance

(NMR), mass spectrometry (MS), and analytical high-performance liquid chromatography (HPLC). Purity was determined to be 99.45% by HPLC analysis. The epoxide was also dissolved directly into serum diluted with untreated serum to achieve the appropriate final concentrations.

At the end of the 48-hr culture period, each embryo having a heartbeat and yolk sac circulation was evaluated using the morphological scoring system of Brown and Fabro ('81) and was measured for crown-rump and head lengths using an ocular micrometer in a Wild M-7A stereomicroscope (Wild Heerbrugg Ltd., Heerbrugg, Switzerland). Embryos were then homogenized by sonication in distilled water; aliquots from individual homogenates were removed for quantitation of DNA using calf thymus DNA as a standard (Labarca and Paigen, '80) and protein (BCA Protein Assay Kit; Pierce, Rockford, IL).

Compound analysis

In order to determine whether CBZ-E is stable during the culture period, the concentration of the epoxide was quantitated in serum using the HPLC method of Taylor et al. ('85). The epoxide was added to culture medium at 48 μg/ml in a flask containing no embryos. These flasks were treated in the same manner as flasks containing embryos (same temperature and gassing protocols). At the end of the 48-hr culture period, the serum was frozen at −20°C until assayed.

To assay for CBZ-E, three volumes of acetonitrile were added to an aliquot of serum in a microfuge tube, vortexed thoroughly and allowed to stand at room temperature for 5 min. The sample was then centrifuged for about 1 min. The supernatant was transferred to a clean microfuge tube and evaporated under a stream of nitrogen gas. The residue was redissolved in acetonitrile, and an aliquot was injected onto the HPLC. The HPLC system consisted of a Waters M500 pump, Rainin injector with a 200-μl injection loop, a Rainin C18 μBondapak column, a Varian UV detector set at 214 nm, and a Hewlett Packard model 3390A integrator. The mobile system was 30% acetonitrile–70% distilled H_2O. A standard curve was established using five different concentrations of the epoxide standard, and the amount of CBZ-E in a sample was determined by comparison to the standard curve.

In order to determine if CBZ-E had reached the embryo, mouse embryos were cultured in serum containing CBZ-E at 48 μg/ml for 48 hr. At the end of the culture period, two embryos were pooled and frozen at −20°C until assayed. The embryos were sonicated in 0.25 ml distilled water. CBZ-E was extracted and assayed as described above. Three groups of pooled embryos were assayed.

Statistical analysis

Data are expressed as mean ±SEM. For most end points, significance was determined by one-way analysis of variance (ANOVA) followed by Duncan's test (Sokal and Rohlf, '69). Differences in the frequencies of embryos with open neural tubes were tested by χ^2 test (Sokal and Rohlf, '69). In all studies, the $P < 0.05$ level of significance was selected.

RESULTS

CBZ had few adverse effects on rat growth and development at concentrations below 60 μg/ml (Table 1). Embryonic DNA content was significantly decreased at nearly all CBZ concentrations tested. All other parameters, with the exception of protein content, were significantly decreased only at the highest CBZ concentration. Mouse embryos appeared to be more sensitive to the embryotoxic effects of CBZ (Table 1). Significant decreases in morphological score, the number of somite pairs, as well as crown-rump and head lengths were observed at all concentrations of CBZ examined (except the number of somite pairs in mouse embryos treated with 24 μg/ml and head length of embryos treated with 60 μg/ml; Table 1). DNA and protein contents were decreased only at the highest CBZ concentration examined. The 10,11-epoxide metabolite of CBZ (CBZ-E) was not embryotoxic to rat or mouse embryos at concentrations as high as 48 μg/ml (Table 2).

Since CBZ treatment has been associated with NTDs in humans, treated rodent embryos were examined closely for adverse effects on neural tube development. Although growth and development of rat embryos were generally not affected by CBZ treatment using the morphological scoring system, there was a dose-related increase in the number of embryos with open anterior neural tubes (Fig. 1). At 24 and 48 μg/ml, the increase in anterior NTDs was due to an open anterior neuropore in the rhombencephalon. Since this is a fairly minor defect, there was no significant decrease in the morphological score of these embryos (Table 1). There was also a dose-related increase in the number of mouse embryos with open anterior neural tubes after treatment with CBZ (Fig. 1). CBZ-E did not significantly increase the incidence of NTDs among rat or mouse embryos (Table 3).

In addition to an open anterior neural tube, CBZ treatment decreased development of the otic and optic systems as well as the forelimb bud and altered flexion in treated mice. Rat embryos treated with the highest concentration of CBZ had decreased development of the branchial arches, otic and optic systems, as well as an altered degree of flexion and open anterior neural tubes.

A possible reason for the lack of embryotoxic effect of CBZ-E could be an instability of the compound in the whole-embryo culture system. The concentration of CBZ-E was measured in serum at the end of the culture period. The initial concentration of 48 μg/ml showed only a slight decline to 47.0 ± 1.9 μg/ml (mean ±SD) after 48 hr, suggesting that the lack of embryotoxicity

TABLE 1. Effect of CBZ on rat or mouse embryonic development in vitro[1]

CBZ Concn (μg/ml)	N	Morphological score[2]	No. somite pairs	Crown-rump length (mm)	Head length (mm)	DNA content[3] (μg/embryo)	Protein content[4] (μg/embryo)
Rats							
0	73	33.8 ± 0.2	23.2 ± 0.2	3.1 ± 0.04	1.6 ± 0.02	12.4 ± 0.5	136 ± 4.8
12	20	33.7 ± 0.3	23.9 ± 0.5	3.1 ± 0.07	1.5 ± 0.03	8.9 ± 0.8*	157 ± 8.4
24	28	31.8 ± 1.0	21.4 ± 0.6*	2.9 ± 0.09*	1.4 ± 0.04*	9.5 ± 0.9*	114 ± 5.9
48	40	32.6 ± 0.6	23.2 ± 0.6	3.0 ± 0.05	1.5 ± 0.02	8.9 ± 0.7*	122 ± 6.3
60	38	29.7 ± 0.9*	20.5 ± 0.6*	2.7 ± 0.08*	1.3 ± 0.04*	7.7 ± 0.6*	123 ± 7.7
Mice							
0	52	36.6 ± 0.5	27.0 ± 0.3	3.4 ± 0.06	1.6 ± 0.04	16.9 ± 0.9	162 ± 6.4
12	15	32.0 ± 1.2*	24.2 ± 0.8*	2.8 ± 0.09*	1.3 ± 0.06*	13.5 ± 0.9	151 ± 8.8
24	16	32.2 ± 1.7*	25.3 ± 0.6	2.9 ± 0.08*	1.4 ± 0.06*	15.5 ± 1.5	134 ± 11.8
48	46	31.2 ± 0.9*	24.2 ± 0.5*	3.0 ± 0.07*	1.4 ± 0.04*	13.6 ± 0.9	150 ± 7.0
60	18	28.7 ± 1.4*	23.8 ± 0.8*	3.0 ± 0.12*	1.5 ± 0.07	12.4 ± 1.5*	129 ± 11.1*

[1]Data presented as means ±SEM.
[2]Determined according to Brown and Fabro ('81).
[3]Determined according to Labarca and Paigen ('80).
[4]Determined using Biorad kit.
*Significantly different from control, $P < 0.05$.

TABLE 2. Effect of CBZ-epoxide on rat or mouse embryonic development in vitro[1]

CBZ-Epoxide Concn (μg/ml)	N	Morphological score[2]	No. of somite pairs	Crown-rump length (mm)	Head length (mm)	DNA content[3] (μg/embryo)	Protein content[4] (μg/embryo)
Rats							
0	49	33.7 ± 0.4	23.0 ± 0.2	3.1 ± 0.04	1.5 ± 0.02	10.2 ± 0.6	128 ± 6.3
8	8	34.0 ± 0.3	22.6 ± 0.6	3.1 ± 0.12	1.6 ± 0.05	12.4 ± 1.4	135 ± 13.9
16	20	32.2 ± 0.9	21.4 ± 0.7	2.9 ± 0.10	1.4 ± 0.05	6.2 ± 0.5*	125 ± 8.5
24	17	32.4 ± 0.4	22.2 ± 0.4	3.0 ± 0.07	1.5 ± 0.04	8.6 ± 1.2	115 ± 5.9
32	20	32.6 ± 0.9	22.9 ± 0.4	3.1 ± 0.08	1.6 ± 0.04	8.5 ± 1.1	144 ± 10.0
48	41	32.7 ± 0.6	21.9 ± 0.5	2.9 ± 0.05	1.5 ± 0.03	8.8 ± 0.5	149 ± 7.2
Mice							
0	32	37.8 ± 0.5	27.9 ± 0.4	3.5 ± 0.06	1.8 ± 0.05	20.5 ± 1.3	188 ± 7.8
8	6	39.3 ± 0.3	28.3 ± 0.2	3.3 ± 0.04	1.6 ± 0.03	16.4 ± 1.2	238 ± 15.0*
12	12	39.1 ± 1.4	26.0 ± 0.9	3.6 ± 0.11	1.9 ± 0.07	22.0 ± 1.8	174 ± 12.3
24	34	35.8 ± 0.8	26.0 ± 0.5	3.3 ± 0.09	1.6 ± 0.06	19.6 ± 0.9	183 ± 11.0
32	11	40.0 ± 0.5	28.7 ± 0.7	3.5 ± 0.12	1.8 ± 0.06	16.2 ± 1.8	183 ± 10.2
48	31	35.7 ± 0.6	25.4 ± 0.3*	3.3 ± 0.06	1.7 ± 0.04	21.9 ± 1.0	195 ± 8.5

[1]Data presented as means ±SEM.
[2]Determined according to Brown and Fabro (1981).
[3]Determined according to Labarca and Paigen (1980).
[4]Determined using Biorad kit.
*Significantly different from control, $P < 0.05$.

of CBZ-E is not due to instability of the compound in culture.

It is also possible that CBZ-E is not taken up by the embryos. The concentration was determined in mouse embryos cultured in 48 μg/ml CBZ-E for 48 hr. Although the level was low (2.65 ± 0.96 μg/embryo, mean ±SD), there was a detectable concentration of CBZ-E in mouse embryos at the end of the culture period, indicating that the compound was taken up by the embryos.

DISCUSSION

CBZ, but not the 10,11-epoxide metabolite, CBZ-E, is embryotoxic in a rodent whole embryo culture system. Mouse embryos were more sensitive than were rat embryos to adverse effects of the parent compound. There was a significant decrease in the morphological score of mouse embryos treated with 12 μg CBZ/ml culture medium, a dose within the human therapeutic range of 3–14 μg/ml (Wilder, '92). Growth and development of rat embryos were only altered at 60 μg/ml, which is well above therapeutic concentrations.

CBZ resulted in a dose-related increase in NTDs among treated mouse embryos (Fig. 1). This malformation has not previously been demonstrated in in vivo studies. Finnell et al. ('86) demonstrated that offspring of mice which consumed CBZ in the diet prior to and throughout gestation had few abnormalities, and no

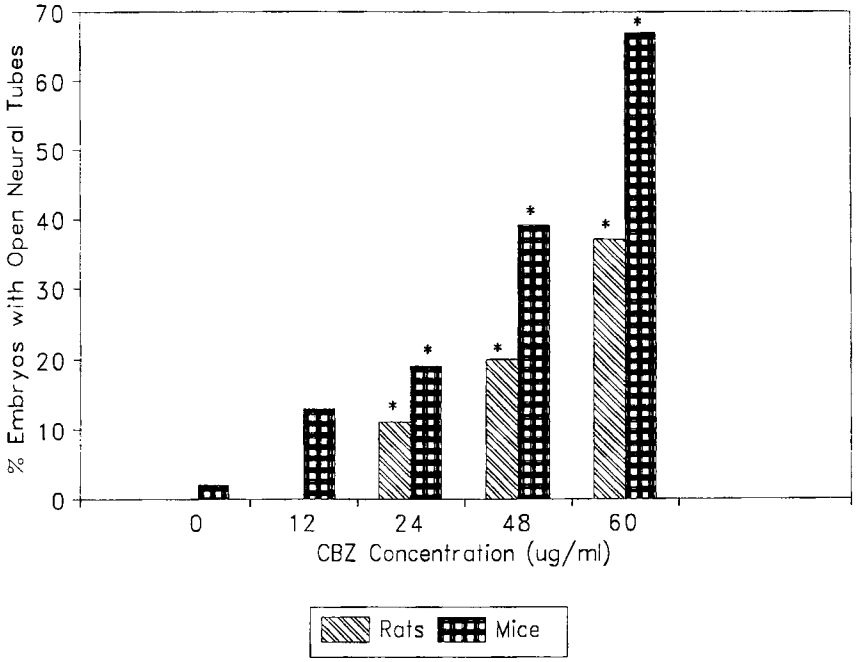

Fig. 1. Frequency of embryos with open neural tubes following treatment with various concentrations of CBZ. CBZ concentrations are shown on the x-axis and the percentage of embryos with open anterior neural tubes on the y-axis. Embryos were treated with CBZ for 48 hr beginning on GD 8 (mice) or GD 9 (rats). *Significantly different from control $P < 0.05$.

TABLE 3. Frequency of open neural tubes following treatment with CBZ-E

Concn (μg/ml)	No. of embryos with NTDs/total No. embryos evaluated	%
Rat embryos		
0	0/49	0.0
8	0/8	0.0
16	2/20	10.0
24	0/17	0.0
32	2/20	10.0
48	3/41	7.3
Mouse embryos		
0	0/38	0.0
8	0/6	0.0
12	0/12	0.0
24	5/34	14.7*
32	0/11	0.0
48	1/31	3.2

*Significantly different from control; $P < 0.05$.

NTDs were reported. In that study, plasma CBZ levels at the end of gestation were 3 μg/ml or less, suggesting very low levels of embryonic exposure to the drug. Finnell et al. ('86) reported higher CBZ-E concentrations than CBZ concentrations; CBZ has been reported to induce its own metabolism (Kerr et al., '94).

When CBZ was administered to mice in the diet on GD 8-13, meningocele and spina bifida were observed, but the incidences were not reported (Paulson et al., '79). The highest plasma drug level reported was 4.26 μg/ml, but it was not clear when this concentration was determined (GD 13 at completion of dosing or GD 18 at sacrifice). When the drug was administered by gavage on GD 8–10 or GD 8–16, no fetuses with NTDs were noted, and no plasma drug levels were reported (Eluma et al., '84). No fetuses with NTDs were observed when CBZ was administered by gavage on GD 6–16 to mice (Sullivan and McElhatton, '77). Although no plasma drug levels were presented, the highest dose administered (240 mg/kg) was much lower than the dietary dose of 1,600 mg/kg used in the Paulson et al. ('79) study, which resulted in a plasma drug level of 4.26 μg/ml. It appears that the doses used in these earlier whole animal studies did not produce plasma drug levels sufficient to produce NTDs.

Since CBZ-E is pharmacologically active, there was concern that this agent might be responsible for embryotoxicity. Evidence of a role for the epoxide came from a study demonstrating that polytherapy resulted in more malformed offspring than did monotherapy (Lindhout et al., '84). The combination of CBZ, phenobarbital and valproic acid produced more malformed offspring than did other combinations of three or four anticonvulsants; the authors suggested that the increase in malformations was due to induction of the cytochrome P-450 system with increased levels of CBZ-E formed. Since the epoxide is stable, it could be formed in the maternal liver and transported to the

embryos. CBZ, CBZ-E, CBZ-diol, and several other metabolites were detected in maternal and cord blood indicating that these compounds can cross the placenta (Pienimäki et al., '95).

However, evidence has been reported suggesting no role for the epoxide metabolite in embryotoxicity. Omtzigt et al. ('93) observed no relationship between the incidence of malformations among exposed human offspring, CBZ or metabolite concentrations (including CBZ-E) or comedication with other anticonvulsants. Finnell et al. ('86) demonstrated that increasing maternal plasma CBZ and CBZ-E concentrations did not influence the incidence of malformations in two inbred strains of mice. The results of the present study also suggest that the epoxide is not embryotoxic.

It is unlikely that CBZ is metabolized to CBZ-E in the fetus. Rodents have very low levels of cytochrome P-450 prenatally, although recent reports suggest that even such low levels of P-450 may be of some consequence (Juchau, '89). Even if it is formed in the embryo, it would appear to be of little importance with respect to embryotoxicity. The formation of CBZ-E appears to result from metabolism by the cytochrome P-450 3A4 isozyme (Kerr et al., '94). This is one of the major P-450 forms found in adult human liver but is not expressed in human fetal liver (Wrighton and Stevens, '92).

It is unknown whether CBZ or another of its metabolites is responsible for embryotoxicity. A number of urinary metabolites have been identified, several of which indicate that other epoxide intermediates might be formed (Lertratanangkoon and Horning, '82). Various lines of evidence suggest that co-oxidation of another anticonvulsant drug, phenytoin, by prostaglandin H synthase may play a role in the embryotoxicity of phenytoin (Wells et al., '89b; Kubow and Wells, '89; Miranda et al., '94). Metabolism by this enzyme system has also been suggested to be involved in embryotoxicity induced by trimethadione (Wells et al., '89a). Further work is needed to determine whether metabolism of CBZ, either by cytochrome P-450 or by prostaglandin H synthase, is involved in embryotoxicity.

ACKNOWLEDGMENTS

We thank Drs. Alenka Tomazic, Alan Walker, and Dwight Miller for synthesis and analysis of CBZ-E under contract 222-92-2000 to the Bionetics Corporation from the National Center for Toxicological Research.

LITERATURE CITED

Brown, N.A., and S. Fabro (1981) Quantitation of rat embryonic development in vitro: A morphological scoring system. Teratology, *24*:65–78.

Dansky, L.V., and R.H. Finnell (1991) Parental epilepsy, anticonvulstant drugs, and reproductive outcome: Epidemiological and experimental finding spanning three decades. 2. Human studies. Reprod. Toxicol., *5*:301–335.

Eichelbaum, M., T. Tomson, G. Tybring, and L. Bertilsson (1985) Carbamazepine metabolism in man. Induction and pharmacogenetic aspects. Clin. Pharmacokinet., *10*:80–90.

Eluma, F.O., M.E. Sucheston, T.G. Hayes, and R.B. Paulson (1984) Teratogenic effects of dosage levels and time of administration of carbamazepine, sodium valproate, and diphenylhydantoin on craniofacial development in the CD-1 mouse fetus. J. Craniofac. Genet. Dev. Biol., *4*:191–210.

Finnell, R.H., V.K. Mohl, G.D. Bennett, and S.M. Taylor (1986) Failure of epoxide formation to influence carbamazepine-induced teratogenesis in a mouse model. Teratogen. Carcinogen. Mutagen., *6*:393–401.

Fujinaga, M., and J.M. Baden (1992) Variation in development of rat embryos at the presomite period. Teratology, *45*:661–670.

Gladstone, D.J., M. Bologa, C. Maguire, A. Pastuszak, and G. Koren (1992) Course of pregnancy and fetal outcome following maternal exposure to carbamazepine and phenytoin: A prospective study. Reprod. Toxicol., *6*:257–261.

Grafton, T.F., J.J. Bazare Jr., D.K. Hansen, and D.M. Sheehan (1987) The in vitro embryotoxicity of 5-fluorouracil in rat embryos. Teratology, *36*:371–377.

Hiilesmaa, V.K., K. Teramo, M.-L. Granström, and A.H. Bardy (1981) Fetal head growth retardation associated with maternal antiepileptic drugs. Lancet, *2*:165–167.

Jones, K.L., R.V. Lacro, K.A. Johnson, and J. Adams (1989) Pattern of malformations in the children of women treated with carbamazepine during pregnancy. N. Engl. J. Med., *320*:1661–1666.

Juchau, M.R. (1989) Bioactivation in chemical teratogenesis. Annu. Rev. Pharmacol. Toxicol., *29*:165–187.

Källén, B. (1986) A register study of maternal epilepsy and delivery outcome with special reference to drug use. Acta Neurol. Scand., *73*:253–259.

Källén, A.J.B. (1994) Maternal carbamazepine and infant spina bifida. Reprod. Toxicol., *8*:203–205.

Kaneko, S., K. Otani, Y. Fukushima, Y. Ogawa, Y. Nomura, T. Ono, Y. Nakane, T. Teranishi, and M. Goto (1988) Teratogenicity of antiepileptic drugs: Analysis of possible risk factors. Epilepsia, *29*: 459–467.

Kerr, B.M., K.E. Thummel, C.J. Wurden, S.M. Klein, D.L. Kroetz, F.J. Gonzalez, and R.H. Levy (1994) Human liver carbamazepine metabolism. Role of cyp3A4 and cyp2C8 in 10,11-epoxide formation. Biochem. Pharmacol., *47*:1969–1979.

Kubow, S., and P.G. Wells (1989) In vitro bioactivation of phenytoin to a reactive free radical intermediate by prostaglandin synthetase, horseradish peroxidase, and thyroid peroxidase. Mol. Pharmacol., *35*:504–511.

Labarca, C., and K. Paigen (1980) A simple, rapid, and sensitive DNA assay procedure. Anal. Biochem., *102*:344–352.

Lertratanangkoon, K., and M.G. Horning (1982) Metabolism of carbamazepine. Drug Metab. Disp., *10*:1–10.

Lindhout, D., R.J.E.A. Höppener, and H. Meinardi (1984) Teratogenicity of antiepileptic drug combinations with special emphasis on epoxidation (of carbamazepine). Epilepsia, *25*:77–83.

Little, B.B., R. Santos-Ramos, J.F. Newell, and M.C. Maberry (1993) Megadose carbamazepine during the period of neural tube closure. Obstet. Gynecol., *82*:705–708.

Miranda, A.F., M.J. Wiley, and P.G. Wells (1994) Evidence for embryonic peroxidase-catalyzed bioactivation and glutathione-dependent cytoprotection in phenytoin teratogenicity: Modulation by eicosatetraynoic acid and buthionine sulfoximine in murine embryo culture. Toxicol. Appl. Pharmacol., *124*:230–241.

Nakane, Y., T. Okuma, R. Takahashi, Y. Sato, T. Wada, T. Sato, Y. Fukushima, H. Kazamatsuri, M. Inami, S. Komai, M. Seino, M. Miyakoshi, T. Tanimura, H. Hazama, R. Kawahara, S. Otsuki, K. Hosokawa, K. Inanaga, Y. Nakazawa, and K. Yamamoto (1980) Multi-institutional study on the teratogenicity and fetal toxicity of antiepileptic drugs: A report of a collaborative study group in Japan. Epilepsia, *21*:663–680.

National Institutes of Health (1985) Guide for the Care and Use of Laboratory Animals. NIH Publ. 85–23.

New, D.A.T. (1978) Whole embryo culture and the study of mammalian embryos during organogenesis. Biol. Rev., *53*:81–122.

Niebyl, J.R., D.A. Blake, J.M. Freeman, and R.D. Luff (1979) Carbamazepine levels in pregnancy and lactation. Obstet. Gynecol., *53*:139–140.

Omtzigt, J.G.C., F.J. Los, J.W.A. Meijer, and D. Lindhout (1993) The 10,11-epoxide-10,11-diol pathway of carbamazepine in early pregnancy in maternal serum, urine, and amniotic fluid: Effect of dose, comedication, and relation to outcome of pregnancy. Ther. Drug Monit., *15*:1–10.

Paulson, R.B., G.W. Paulson, and S. Jreissaty (1979) Phenytoin and carbamazepine in production of cleft palates in mice. Arch. Neurol., *36*:832–836.

Pienimäki, P., A.-L. Hartikainen, P. Arvela, T. Partanen, R. Herva, O. Pelkonen, and K. Vähäkangas (1995) Carbamazepine and its metabolites in human perfused placenta and in maternal and cord blood. Epilepsia, *36*:241–248.

Rosa, F.W. (1991) Spina bifida in infants of women treated with carbamazepine during pregnancy. N. Engl. J. Med., *324*:674–677.

Schardein, J.L. (1993) "Anticonvulsants" In: Chemically Induced Birth Defects. Marcel Dekker, New York, pp. 157–207.

Scialli, A.R., and A. Lione (1989) Teratogenic effects of carbamazepine. N. Engl. J. Med., *321*:1480–1481.

Scolnik, D., I. Nulman, J. Rovet, D. Gladstone, D. Czuchta, H.A. Gardner, R. Gladstone, P. Ashby, R. Weksberg, T. Einarson, and G. Koren (1994) Neurodevelopment of children exposed in utero to phenytoin and carbamazepine monotherapy. JAMA, 271:767–770.

Sillanpää, M. (1993) "Carbamazepine" In: The Treatment of Epilepsy: Principles and Practices. E. Wyllie, ed. Lea & Febiger, Philadelphia, pp. 867–886.

Sokal, R.R. and F.J. Rohlf (1969) Biometry. W.H. Freeman, San Francisco.

Sullivan, F.M., and P.R. McElhatton (1977) A comparison of the teratogenic activity of the antiepileptic drugs carbamazepine, clonazepam, ethosuximide, phenobarbital, phenytoin, and primidone in mice. Toxicol. Appl. Pharmacol., *40*:365–378.

Taylor, S.M., G.D. Bennett, L.C. Abbott, and R.H. Finnell (1985) Seizure control following administration of anticonvulsant drugs in the quaking mouse. Eur. J. Pharmacol., *118*:163–170.

Tomson, T., U. Lindbom, B. Ekqvist, and A. Sundqvist (1994) Disposition of carbamazepine and phenytoin in pregnancy. Epilepsia, *35*: 131–135.

Vorhees, C.V., K.D. Acuff, W.P. Weisenburger, and D.R. Minck (1990) Teratogenicity of carbamazepine in rats. Teratology, *41*:311–317.

Wells, P.G., M.K. Nagai, and G.S. Greco (1989a) Inhibition of trimethadione and dimethadione teratogenicity by the cyclooxygenase inhibitor acetylsalicylic acid: A unifying hypothesis for the teratogenic effects of hydantoin anticonvulsants and structurally related compounds. Toxicol. Appl. Pharmacol., *97*:406–414.

Wells, P.G., J.T. Zubovits, S.T. Wong, L.M. Molinari, and S. Ali (1989b) Modulation of phenytoin teratogenicity and embryonic covalent binding by acetylsalicylic acid, caffeic acid, and α-phenyl-*N*-*t*-butylnitrone: Implications for bioactivation by prostaglandin synthetase. Toxicol. Appl. Pharmacol., *97*:192–202.

Wilder, B.J. (1992) Pharmacokinetics of valproate and carbamazepine. J. Clin. Psychopharmacol., *12*:564–568.

Wrighton, S.A., and J.C. Stevens (1992) The human hepatic cytochromes P450 involved in drug metabolism. Crit. Rev. Toxicol., *22*:1–21.