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Report

Dose-Dependent Pharmacokinetics of Carbamazepine in Rats: Determination of the Formation Clearance of Carbamazepine-10,11-epoxide[1]

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The dose dependency of carbamazepine pharmacokinetics was characterized in rats, a common test animal for antiepileptic drug efficacy. With a randomized Latin square schedule, 5, 10, and 20 mg/kg doses of carbamazepine were injected intravenously into six Sprague-Dawley rats followed by the administration of a 5 or 10 mg/kg i.v. dose of CBZ-E to each rat. Following administration, the concentrations of CBZ and carbamazepine-10,11-epoxide (CBZ-E) in whole blood were determined by a reverse-phase HPLC assay. Plasma protein binding of both carbamazepine and CBZ-E was linear over the concentration range observed in this study. Carbamazepine concentration–time plots were log-linear, but the slopes were not parallel. Carbamazepine total-body clearances were 15.1 ± 3.26, 13.4 ± 5.66, and 10.0 ± 3.11 ml/min/kg at the 5, 10, and 20 mg/kg doses, respectively (significance of difference between the 5 and the 20 mg/kg dose = $0.06 < P < 0.05$; Kruskal–Wallis test, Dunn's procedure). However, the formation clearance to CBZ-E did not change, suggesting that metabolism via other pathways was decreased at higher carbamazepine doses.

KEY WORDS: carbamazepine; carbamazepine-10,11-epoxide; pharmacokinetics; dose dependency; metabolite; blood.

INTRODUCTION

Studies on the dose dependency of carbamazepine in patients have yielded conflicting results (1–5). In the present report, the pharmacokinetics of carbamazepine and its active metabolite, carbamazepine-10,11-epoxide (CBZ-E), were studied in rats which are common laboratory animals used for testing of antiepileptic drugs.

Carbamazepine is extensively metabolized via several pathways including epoxidation at the 10,11 double bond (6). Carbamazepine-10,11-epoxide is further metabolized to carbamazepine-10,11-trans-diol via epoxide hydrolase (6). The epoxide-diol pathway is responsible for 33–61% of the urinary excretion in patients on other antiepileptic drugs, e.g., primidone and phenytoin, but accounts for only 22–29% of the urinary excretion in patients on monotherapy (7). Rats excrete a higher proportion of unchanged CBZ-E in the urine compared to humans, apparently because of a low level of epoxide hydrolase (8). CBZ-E prevents maximal electroshock seizures in mice at brain concentrations 1.5 times higher than effective brain concentrations of carbamazepine. Neurotoxicity (rotorod test) was observed at equivalent brain concentrations for both compounds, while carbamazepine-10,11-diol was inactive in both tests (9). In humans, CBZ-E is as effective as carbamazepine in treating trigeminal neuralgia (10).

Several groups have studied the pharmacokinetics of carbamazepine in the rat, a commonly used test animal in dose–response studies, e.g., in response to pentylenetetrazol-, allyl glycine-, or cefazolin-induced seizures, and in antiepiletic drug interaction studies (11–14). None of these studies examined the dose dependency of carbamazepine disposition in rats at clinically relevant concentrations, which is the subject of this report.

MATERIALS AND METHODS

Chemicals

Carbamazepine was purchased from Sigma Chemical Co. (St. Louis, MO). Carbamazepine-10,11-epoxide was synthesized from carbamazepine by the procedure of Bellucci *et al.* (15). Cyheptamide, the internal standard, was purchased from Supelco (Bellefonte, PA). Carbamazepine and CBZ-E were prepared at concentrations of 5 and 10 mg/ml in 60% polyethylene glycol-400 (v/v). Sodium pento-

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barbital was obtained from Abbott Laboratories (Chicago, IL). Spectrosocopic-grade dichloromethane and methanol were obtained from Mallinckrodt (Paris, KY). HPLC-grade acetonitrile was from Fisher Scientific (Fair Lawn, NJ). HPLC-grade ammonium phosphate was obtained from J. T. Baker (Phillipsburg, NJ). All other chemicals were reagent grade.

Surgical Procedure

Male Sprague–Dawley rats (250–325 g) were anesthesized with sodium pentobarbital (45 mg/kg i.p.). The jugular and femoral veins were cannulated with 0.020- and 0.025-in.-i.d. Silastic tubing (Dow-Corning, Midland, MI) connected to PE-50 polyethylene tubing via a 1-cm piece of 23-gauge needle. The PE-50 tubing exited behind the animal's head and was protected by a tether and swivel apparatus (Harvard Bioscience, South Natick, MA). Each rat was allowed 48 hr to recover from surgery. The animals were housed in plastic cages on corn-cob bedding with a 12-hr light/dark cycle and allowed food (Purina Rat Chow, St. Louis, MO) and water ad libitum. On the third day, the animals were weighed and a bolus dose of carbamazepine was administered over 30 sec through the jugular vein cannula. Blood (approximately 150 μl) was collected in 2-ml vacutainer tubes containing sodium heparin at 2, 15, 30, 60, 90, 120, 180, 240, 300, and 360 min and at 8, 10, and 12 hr, depending on the amount of bolus dose (5, 10, or 20 mg/kg, respectively). The two remaining doses were administered on the fifth and seventh days. The dosing schedule, which was randomized in a Latin square design for the individual rats, was as follows: Rat A = 5–10–20, Rat B = 20–10–5, Rat C = 20–5–10, Rat D = 10–5–20, Rat E = 5–20–10, and Rat F = 10–20–5. A randomized design was employed to minimize effects caused by treatment order or by possible autoinduction. On the ninth day after surgery, an intravenous dose of CBZ-E was given to rats B through F and blood was collected on a similar schedule to that described above for carbamazepine. Rats C, D, and F were administered 5 mg/kg of carbamazepine-epoxide and Rats B and E were administered 10 mg/kg of carbamazepine-epoxide. Aliquots (100 μl) of anticoagulated blood were removed into separate tubes and stored at −20°C until analysis.

Plasma Protein Binding

To determine if the protein binding of carbamazepine was concentration dependent, the fraction unbound in rat plasma was determined at 1, 5, and 10 μg/ml of carbamazepine. Fresh blood (10 ml) was obtained from a 400-g rat under ether anesthesia. Carbamazepine was dissolved in methanol and aliquots equivalent to 0.5, 2.5, and 5.0 μg were placed in test tubes (three tubes per concentration) and evaporated to dryness. Plasma (0.5 ml) was added to each tube and the tubes were heated to 37°C to dissolve the carbamazepine. An aliquot of the plasma (100 μl) was removed to determine total concentration and the remaining 400 μl was placed in an Amicon Centrifee ultrafiltration device (Danvers, MA). The ultrafiltration device was centrifuged for 20 min at 1500*g* and 100 μl of the filtrate was analyzed for carbamazepine content. To determine whether surgical manipulation affected protein binding, blood was also obtained from a control rat with implanted femoral and jugular vein cannulas. In this experiment, whole blood was spiked with carbamazepine, and the concentrations of carbamazepine in whole blood, plasma, or ultrafiltrate were determined by HPLC. To determine if CBZ-E affected the plasma binding of carbamazepine, carbamazepine-10,11-epoxide was also added to the blood from the same cannulated rat to obtain final blood concentrations of 5, 10, and 20 μg/ml along with carbamazepine at 10 μg/ml. The blood/plasma ratios and plasma protein binding were determined in duplicate at each concentration as described above for both compounds.

Analysis

The analytical procedure was similar to the procedure of Sawchuk and Cartier (16) with the following modifications. Whole blood (100 μl) was mixed with 100 μl of 0.01 *M* sodium phosphate buffer, pH 7.8. Dichloromethane (4 ml) containing 0.25 μg/ml of cyheptamide was added to the sample and the mixture was vortexed for 30 sec. The samples were centrifuged for 5 min at 1500*g* and the aqueous layer was removed by aspiration. The organic layer was evaporated under N_2 at 40°C and reconstituted in 100 μl of mobile phase. An aliquot (40 μl) was injected onto a 150 × 4.6-mm-i.d. Spherisorb C8 cartridge column protected by a 10 × 4.6-mm Adsorbosphere C8 guard cartridge (Alltech Associates, Deerfield, IL). The mobile phase consisted of acetonitrile/methanol/0.01 *M* ammonium phosphate, pH 7.5 (29:7:64, v/v). The flow rate was 1.5 ml/min at ambient temperature. The solvent delivery system was a Spectra-Physics SP8700 with a Rheodyne 7125 injector (Santa Clara, CA) and the compounds were detected with a Waters Model 450 UV detector (Milford, MA) set at 212 nm. Peak height ratios were used to determine the concentrations of carbamazepine and CBZ-E as measured by a Shimadzu CR3A integrator (Columbia, MD). Standard curves ranging from 0.1 to 10 μg/ml were prepared in duplicate for each analysis day. Between-day variability for carbamazepine ranged from 13% at 0.1 μg/ml to 4.5% at 10 μg/ml. The coefficient of variation was less than 7% at all concentrations for both carbamazepine and CBZ-E except at 0.1 μg/ml.

Pharmacokinetic and Statistical Analyses

The area under the curve (AUC) was determined by the trapezoidal rule. The terminal area for carbamazepine was determined by triangulation with the slope of the regressed line. The terminal AUC of CBZ-E resulting from a carbamazepine dose was determined by triangulation by multiplying the last measured concentration (8, 10, or 12 hr postdose) with the slope from the i.v. bolus dose of CBZ-E. The total-body clearance (Cl) was calculated by dividing the administered dose by the AUC. The fraction of carbamazepine metabolized to CBZ-E was determined by dividing the AUC of the metabolite after a dose of carbamazepine by the AUC from a CBZ-E dose and adjusting for the appropriate molar dose. Formation clearance was determine by multiplying the fraction metabolized by the total body clearance for carbamazepine. The assumptions for the determination of the fraction metabolized (formation clearance) are as follows. (i) The pharmacokinetics of CBZ-E are not concentration dependent within the range of measured concentrations. (ii) Clear-

ance of CBZ-E was constant during the three doses of carbamazepine and the single dose of CBZ-E. (iii) The three doses of carbamazepine did not change (induce) the metabolism of CBZ-E. (iv) Plasma protein binding of both carbamazepine and CBZ-E was constant in all four dosage regimens. Statistical analyses were done by the nonparametric Kruskal–Wallis test. For contrasts between doses, the Dunn procedure was employed.

RESULTS

The decline in carbamazepine concentrations in blood was log-linear at all three doses and followed first-order elimination kinetics. However, it was apparent that the slopes of the regression lines for the concentration–time data were different among the three doses (Fig. 1A), and the data did not display Michaelis–Menten characteristics. Figure 1B shows the CBZ-E concentrations in whole blood after administration of carbamazepine to the rats. The individual pharmacokinetic data are presented in Table I for the six rats. Clearance decreased with increasing dose with a reduction of clearance of approximately 33% between the 5 and the 20 mg/kg dose. A weak, but significant, linear correlation exists between dose and clearance ($r = -0.485, P < 0.05$, df = 16). Calculations of volume of distribution were not done because both compartmental and noncompartmental models assume linearity for the determination of volume. However, the decrease in clearance at higher doses was directly proportional to the increase in the apparent half-life for carbamazepine, suggesting that volume was not affected greatly by changes in dose.

The pharmacokinetic data for CBZ-E in rats B–F are summarized in Table II, and the pharmacokinetic profile of a representative rat is shown in Fig. 2. After intravenous administration of carbamazepine, the concentrations of the epoxide peaked in 1–2 hr and declined more slowly than carbamazepine, indicating a longer half-life of CBZ-E compared to carbamazepine in rats (see Fig. 1B). The percentage of the total AUC of CBZ-E produced after a dose of CBZ estimated by triangulation by using the slope of the i.v. bolus

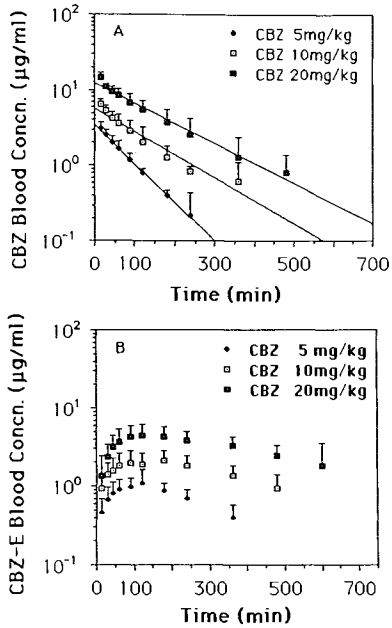

Fig. 1. The log-concentration–time course of carbamazepine (A) andcarbamazepine-10,11-epoxide (B) after intravenous doses of 5, 10, or 20 mg/kg of carbamazepine. The lines represent simple linear regressions of the carbamazepine data points. Data points and errors bars represent mean whole-blood concentrations and standard deviations, respectively, collected from six rats.

Table I. Pharmacokinetic Parameters[a] of Carbamazepine at Doses of 5, 10, and 20 mg/kg

Rat	AUC	Cl	k_e
	5 mg/kg dose		
A	238	21.0	0.0186
B	348	14.4	0.0172
C	329	15.2	0.0064
D	419	11.9	0.0052
E	322	15.6	0.0080
F	403	12.4	0.0063
Mean	343	15.1	0.0103
SD	64.9	3.26	0.0060
	10 mg/kg dose		
A	429	23.3	0.0160
B	805	12.4	0.0080
C	934	10.7	0.0072
D	907	11.0	0.0023
E	1441	6.94	0.0059
F	627	15.9	0.0125
Mean	857	13.4	0.0087
SD	343	5.66	0.0049
	20 mg/kg dose		
A	1718	11.6	0.0116
B	2124	9.42	0.0048
C	1428	14.6	0.0050
D	2315	8.64	0.0030
E	3784	5.29	0.0040
F	1893	10.6	0.0067
Mean	2210	10.0*	0.0059
SD	831	3.11	0.0031

[a] Units for the various parameters are AUC = µg-min/ml-kg; Cl = ml/min-kg; and k_e = min^{-1}.

* $0.06 < P < 0.05$, compared to the 5 mg/kg dose; Kruskal–Wallis test, Dunn's procedure.

Table II. Pharmacokinetic Parameters of Carbamazepine-10,11-epoxide After Intravenous Administration

Rat	CBZ-E dose (mg/kg)[a]	AUC	Cl	k_e
B	10	2122	4.71	0.0035
C	5	713	7.01	0.0053
D	5	1317	3.80	0.0040
E	5	633	7.90	0.0055
F	10	2964	3.37	0.0031
Mean			5.36	0.0043
SD			2.00	0.0011

[a] A single i.v. bolus dose of CBZ-E was administered to Rats B–F, 2 days after completion of three doses of carbamazepine (see Table I).

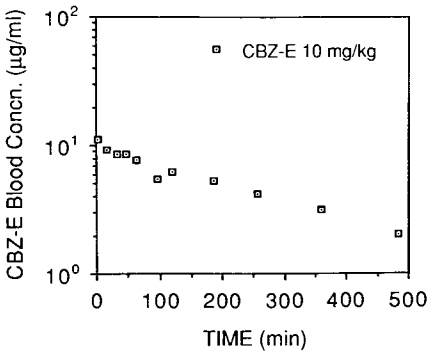

Fig. 2. The log-concentration–time course of carbamazepine-10,11-epoxide after a 10 mg/kg intravenous dose of carbamazepine-10,11-epoxide to Rat F, 2 days after completion of the randomized carbamazepine dose schedule.

dose of CBZ-E was 13.0 ± 5.16, 12.9 ± 11.0, and 24.0 ± 16.0% at the 5, 10, and 20 mg/kg doses, respectively. The total AUC of CBZ-E from the 10 and 20 mg/kg doses may have been slightly underestimated if formation of CBZ-E occurred after the last measured time point, but the difference would be small considering the total contribution of the extrapolated area and the observation that greater than 90% of the total carbamazepine AUC was accounted for by the last time point at each dose. Table II shows that CBZ-E has a lower clearance than the parent drug, with an estimated mean half-life of 161 min after an intravenous dose compared to the half-lives of carbamazepine (67, 80, and 117 min at the three doses). The fractions metabolized and the formation clearance are shown in Table III. As the dose increased, the formation clearance of CBZ-E was unchanged, although the overall clearance of carbamazepine decreased by 33%.

Carbamazepine plasma protein binding remained constant over the range of concentrations observed in this study. The percentage bound in plasma from an untreated rat was 68.6 ± 1.67% at 1 μg/ml, 70.3 ± 0.69% at 5 μg/ml, and 68.9 ± 1.21% at 10 μg/ml (n = 3). These values are similar to the 68 and the 75% protein bound in rats as reported by Schmutz (17) and Chang and Levy (13), respectively, when determined by equilibrium dialysis. The plasma protein binding of carbamazepine in a second rat 48 hr after surgical manipulation was 58.0 ± 3.72% in a concentration range of 1–10 μg/ml (n = 5). The presence of CBZ-E at concentrations of 5, 10, or 20 μg/ml did not affect the binding of carbamazepine (10 μg/ml) in plasma (58.9 ± 4.73% of carbamazepine bound; n = 5). The plasma protein binding of the CBZ-E (5–20 μg/ml) in the presence of 10 μg/ml of CBZ was 30.7 ± 3.12%. The blood/plasma ratios were 1.37 ± 0.125 (n = 5) and 1.32 ± 0.134 (n = 6) for carbamazepine and CBZ-E, respectively.

DISCUSSION

The clearance values reported in this study were in the same range as those previously observed in rats (12–14). Chang and Levy (13) reported a formation Cl of CBZ-E of 8.0 ± 2.2 ml/min/kg (0.51 fraction metabolized) in rats in their steady-state experiments, which is close to the fraction metabolized to CBZ-E after a 20 mg/kg bolus dose found in these experiments. In this study, plasma concentrations of active compounds (carbamazepine plus CBZ-E) at any time point were not proportional to dose. If one assumes that unbound plasma concentrations correspond to a pharmacologically active concentrations in the brain, then determination of pharmacological response and toxicity will not be directly proportional to dose in rats.

Following a single dose, the clearance of carbamazepine in rats decreases with increasing dose at concentrations that are in the therapeutic range in humans. The mechanism by which carbamazepine clearances decrease as doses increase is not obvious. It does not appear to be a typical Michaelis–Menten saturation process because the slope of the disappearance is constant throughout the measured concentration range for a given dose. As an example, phenytoin displays saturation kinetics both in rats and in man and has a concentration–time profile that is log-curvilinear (Michaelis–Menten) (18). Plasma protein binding of carbamazepine is not concentration dependent and is also not affected by CBZ-E concentration in the rat. Therefore, plasma binding does not appear to contribute to the dose-dependent changes.

As the clearance of carbamazepine decreased with larger doses, a greater proportion of the dose was metabolized to the epoxide (see Tables I and III). However, the

Table III. Effect of Dose on the Formation Clearance of Carbamazepine-10,11-epoxide

	5 mg/kg dose			10 mg/kg dose			20 mg/kg dose		
Rat	$AUC_{cbz\text{-}e/cbz}$[a]	f_m[b]	Cl_f[c]	$AUC_{cbz\text{-}e/cbz}$	f_m	Cl_f	$AUC_{cbz\text{-}e/cbz}$	f_m	Cl_f
B	467	0.41	5.90	1377	0.61	7.56	3147	0.79	6.50
C	230	0.30	4.56	584	0.38	4.07	1814	0.60	8.76
D	735	0.52	6.19	1675	0.59	6.49	4471	0.79	6.85
E	278	0.41	6.40	410	0.30	2.08	1395	0.52	2.75
F	308	0.19	2.36	1416	0.45	7.16	2066	0.33	3.50
Mean	403	0.37	5.08	1092	0.47	5.47	2579	0.58	5.67
SD	205	0.12	1.68	558	0.13	2.33	1240	0.18	2.49

[a] The areas-under-the-curve of CBZ-E produced from i.v bolus doses of carbamazepine ($AUC_{cbz\text{-}e/carbamazepine}$) were determined from the whole-blood concentrations in rats B–F (see Table I).

[b] The fractions metabolized (f_m) were determined by dividing $AUC_{cbz\text{-}e/carbamazepine}$ by AUC of CBZ-E from a bolus dose of CBZ-E (see Table II) and correction for the molar dose.

[c] The formation clearance (Cl_f) of CBZ-E. Determined by multiplying f_m by the Cl of carbamazepine.

formation clearance of CBZ-E was not changed by increasing dose (Table III). Given the inherent variability in the clearance among rats (20–40% coefficient of variation), these data suggest that some other metabolic pathway of carbamazepine is inhibited, resulting in a decreased clearance of the parent compound at a dose of 20 mg/kg compared to a 5 mg/kg dose. Two possible mechanisms could explain these data: (i) saturation of some other metabolic pathway, e.g., aromatic hydroxylation (although saturation kinetics were not observed) and (ii) product inhibition of other metabolic pathways, perhaps by CBZ-E, the major circulating metabolite in rats.

Further support for nonsaturation of the epoxide-diol pathway comes from the data of Regnaud *et al.* (19). The excretion of CBZ-E and carbamazepine-10,11-diol in rats were approximately equal and accounted for 7–10% of the dose in urine after 24 hr. The percentage of the recovered metabolites excreted by the epoxide-diol pathway was not affected by increasing i.p. doses of 30, 60, or 100 mg/day, but unchanged carbamazepine increased from less than 1% of the recovered metabolites at 30 mg/kg to 20% of the recovered metabolites at 100 mg/kg (19). Their data also indicate that saturation or inhibition of metabolizing enzymes occurred at the higher doses.

Recently, Sumi *et al.* investigated the dose dependency of carbamazepine in rabbits at 5, 10, 15, and 20 mg/kg (20). Clearance was constant at doses less than 20 mg/kg. The Cl was slightly lower at a 20 mg/kg dose. The fraction metabolized to the epoxide in rabbits was 0.152 ± 0.055 for a 10 mg/kg dose of carbamazepine and a 5 mg/kg dose of CBZ-E. The Cl of CBZ-E displayed marked nonlinearity at doses greater than 10 mg/kg (lower Cl at higher doses). In comparison to our data in rats, rabbits have a higher Cl at 10 mg/kg than rats (20.3 ± 7.83 mg/min/kg in rabbits vs 13.9 ± 5.71 ml/min/kg in rats). However, the fraction metabolized to the epoxide in rabbits is lower than in rats. Rabbits also display a dose-dependent decrease in clearance at doses of 20 mg/kg or greater, which may indicate saturation or inhibition of metabolizing enzymes at higher carbamazepine concentrations in rabbits.

These studies indicate that care must be taken in the design and interpretation of future dose–response studies with carbamazepine in rats. Response should be related directly to measured unbound concentrations (or CSF concentrations) of carbamazepine and CBZ-E rather than dose. Antiepileptic drug interaction studies in animals should also take into account the observations described in this report.

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