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Report

Evaluation of the Pharmacokinetic Interaction Between Diazepam and ACC-9653 (a Phenytoin Prodrug) in Healthy Male Volunteers[1]

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The protein binding and pharmacokinetics of diazepam, ACC-9653 (a phenytoin prodrug), and phenytoin were evaluated in nine healthy male volunteers following administration of diazepam and ACC-9653, alone or concomitantly, in a randomized crossover design. No significant differences were observed in the fraction unbound or pharmacokinetic parameters of ACC-9653, phenytoin, or diazepam when ACC-9653 was administered alone compared to concomitant administration with diazepam. The phenytoin fraction unbound increased significantly with increased concentrations of ACC-9653, indicating displacement of phenytoin from its binding sites by ACC-9653. ACC-9653 also demonstrated concentration dependent binding. The lack of a significant pharmacokinetic drug interaction between ACC-9653 and diazepam suggests that these drugs may be safely administered together, although this conclusion should be confirmed in the intended patient population.

KEY WORDS: diazepam; phenytoin; ACC-9653; prodrug; protein binding; pharmacokinetics.

INTRODUCTION

Simultaneous intravenous administration of diazepam and phenytoin is currently recommended for management of status epilepticus (1,2). Intravenous diazepam generally stops convulsions within 3–5 min after intravenous administration (2). Ten to twenty minutes after diazepam administration seizures may recur due to the rapid distribution of diazepam from blood. Anticonvulsant effects of phenytoin are not evident until 10 to 20 min after the start of phenytoin infusion, probably because of the slow recommended rate of infusion (not to exceed 50 mg/min). Phenytoin is relatively water insoluble and is formulated in 40% propylene glycol. Limitations associated with intravenous administration of the marketed phenytoin product include its incompatibility with iv solutions and a basic pH of 12, which is associated with a high incidence of phlebitis, including pain at the injection site (3,4).

ACC-9653 (disodium phosphate ester of 3-hydroxymethyl-5,5-diphenylhydantoin) is a water-soluble ester prodrug of phenytoin with a pH of 8.8. ACC-9653 is rapidly converted to phenytoin by nonspecific esterases (mean half-life of 8.1 min) following iv or im injection (5). The pharmacokinetics of ACC-9653 have been evaluated in healthy male volunteers receiving single iv or im doses of 150 to 1200 mg. Conversion of ACC-9653 to phenytoin following parenteral ACC-9653 administration is complete. Jamerson *et al.* (6) demonstrated that the phenytoin fraction unbound was significantly higher (9.6 vs 8.4% at the end of a 30-min infusion) when ACC-9653 was administered as compared to when phenytoin was administered. No difference was observed in the phenytoin fraction unbound 3 hr after the administration of either drug. This finding was attributed to displacement of phenytoin by the prodrug. The objective of the current study was to determine the extent of displacement of phenytoin, as well as the ACC-9653 fraction unbound, following iv administration of ACC-9653. Additionally, because of the prevalent clinical use of the highly protein bound drug, diazepam in the initial treatment of status epilepticus, and the likelihood of concomitant diazepam and ACC-9653 administration, a secondary objective of this study was to investigate potential pharmacokinetic and protein binding interactions among ACC-9653, phenytoin, and diazepam.

METHODS

Healthy, nonsmoking male volunteers were recruited. A medical history, physical examination, 12-lead electrocardiogram (EKG), blood chemistry, hematology, urinalysis, and urine screen for drugs of abuse were performed and subjects were excluded if significant abnormal values were

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noted. The study was approved by the Committee on the Protection of the Rights of Human Subjects of the School of Medicine, University of North Carolina, Chapel Hill. Informed consent was obtained prior to study initiation.

The first treatment was administered within 2 weeks of the screening examination. The evening prior to administration of the study drug, the subject was admitted to the Clinical Research Unit at the North Carolina Memorial Hospital. Vital signs were obtained and the subject was interviewed to ascertain compliance with study criteria. Subjects were not allowed to consume alcoholic beverages, take medications without the approval of the investigators, or use tobacco products throughout the entire study period. Each subject remained an inpatient until after the 24-hr blood sample was obtained.

On the morning of the study, between 7 and 8 AM, each subject received the following treatments in a randomized Latin square design:

(A) 1125 mg ACC-9653 (Dupont) iv over 15 min (1125 mg/15 ml), beginning 15 min after the start of the study, equivalent to a total dose of 750 mg phenytoin;

(B) 10 mg diazepam (Valium, Roche) iv over 5 min followed by 1125 mg ACC-9653, as above (ACC-9653 infusion began 10 min after the end of the diazepam infusion); and

(C) 10 mg diazepam (Valium, Roche) iv over 5 min (2 mg/min; 5 mg/ml).

Both drugs were administered by a Harvard infusion pump at the specified rate.

Safety and tolerance assessments were obtained frequently throughout the initial sampling period. A continuous EKG was obtained for the duration of each drug infusion and every 5 min for the first hour.

Each drug was administered at a separate injection site in the same arm through a 19-gauge butterfly needle which was removed after the 60-min blood sample was collected. The point of needle entry into the vein was marked for each infusion site. Subjects were asked if they felt pain, burning, or itching, the severity of which was rated on a scale of 1 to 10 (10 being the most severe). Additionally, the injection site was evaluated for erythema, swelling, tenderness, induration, peeling/flaking, exudation, phlebitis, cording, sclerosis, and necrosis. Each infusion site was evaluated before the infusion and at 5 and 30 min and 4, 8, 24, 48, and 72 hr post-infusion.

All blood samples were collected relative to the scheduled time of diazepam administration (0–5 min), even during the one treatment when only ACC-9653 was administered. Blood samples were collected in heparinized vacuum tubes (Vacutainer, Becton-Dickinson) at 0, 20, 30, 45, 60, and 90 min and at 2, 4, 8, 12, 24, 48, and 72 hr after the start of the diazepam infusion for determination of diazepam, phenytoin, and ACC-9653 plasma concentrations. In addition, blood was obtained at 35, 40, 50, 55, 75, and 105 min only when ACC-9653 was administered and at 5 min when diazepam was administered for determination of the respective plasma concentrations. Additional blood for the determination of the fraction unbound for diazepam, phenytoin, and ACC-9653 was obtained at 0, 20, 30, 45, 60, and 90 min and at 2, 8 and 12 hr after the scheduled start time of the diazepam infusion. When diazepam was administered, a sample was also obtained at 5 min for the determination of the fraction unbound. Each sample was placed on ice and immediately centrifuged at 4°C (3000 rpm) for 10 min to separate the plasma.

Plasma was immediately processed to determine the fraction unbound of diazepam, phenytoin, and ACC-9653 by ultrafiltration using an Amicon (Centrifree-TM) micropartition system (W. R. Grace & Co., Danvers, MA). Methyl-^{3}H-diazepam (New England Nuclear, Boston, MA) was diluted in ethanol and 0.923 μCi per sample was used in protein binding determinations. Radiochemical purity was confirmed to be greater than 97.7% by thin-layer chromatography on Silica Gel. Radiochemical purity of ^{3}H-phenytoin and ^{14}C-ACC-9653 (Dupont Critical Care) was confirmed to be greater than 99 and 97.9%, respectively, by thin-layer chromatography. For protein binding determinations, 0.46 μCi ^{3}H-phenytoin and 0.394 μCi ^{14}C-ACC-9653 were used per sample. Determination of the fraction unbound for phenytoin (7–9) and diazepam (10–13) has been described previously. Each Amicon device was centrifuged (2000 rpm) at room temperature for 8 min (determined as the time to produce a 10% yield of the initial volume as filtrate) in a fixed-angle rotor. A liquid scintillation counter was used to determine the counts per minute in filtrate and plasma. All samples were processed in duplicate and the mean fraction unbound was determined for each time point as the ratio of the filtrate to plasma disintegrations per minute. Adsorption to the Amicon system was found to be <3.1% for ^{3}H-phenytoin and ^{14}C-ACC-9653 and <11% for ^{3}H-diazepam. Within- and between-day coefficients of variation for protein binding determinations were 8.9 and 9.8% for phenytoin, 5.6 and 7.6% for ACC-9653, and 9.3 and 11.5% for diazepam, respectively.

Once the plasma was separated, 1.0 ml was immediately added in duplicate to a glass tube containing 0.1 ml 5-(4-methylphenyl)-5-phenylhydantoin (20 μg/ml) and 0.1 ml diphenyl phosphate (50 μg/ml) as internal standards. Additionally, each tube contained 0.1 ml water and 3.0 ml acetonitrile. The tube was immediately mixed and centrifuged for 10 min at 3000 rpm. The supernatant was decanted to a glass tube and frozen until shipped to Dupont for analysis of ACC-9653 and phenytoin concentrations by HPLC (5).

Plasma samples for determination of the diazepam concentration were stored at −70°C for no more than 6 months. Diazepam concentrations were determined by modification of diazepam assays previously described (14–16). Components of the HPLC system included a Spherisorb S5ODS2 ¼-in.-o.d., 4.9 mm × 25-cm reverse-phase column and Waters (Model 480) variable-wavelength (254-nm) LC spectrophotometer (0.002–2.0 AUFS). The mobile phase consisted of 10% methanol, 45% acetonitrile, and 45% 7 m*M* K_2HPO_4 buffer adjusted to pH 3.0 with 85% phosphoric acid flowing at a rate of 2.0 ml/min. The retention times for diazepam (Hoffman La Roche, Nutley, NJ) and the internal standard, prazepam (Warner Lambert Co., Ann Arbor, MI), were 7 and 12 min, respectively. Diazepam was extracted from plasma by a Baker 10-Extraction System with disposable columns (C-18, 40-μm 60A silica, 1-ml capacity; J.T. Baker Research Products, Phillipsburg, NJ). Following column preconditioning, 100 μl of prazepam stock solution (40

μg/ml in 0.1 M Na_2CO_3) and the plasma sample (0.5 ml) were added to each column and extracted, as previously described (15). Each sample was extracted and analyzed in duplicate and mean values are reported. The extraction efficiency was >82.6% over concentrations ranging from 20 to 1200 ng/ml. Assay sensitivity under these conditions was 20 ng/ml of extracted sample. Within-day coefficients of variation were 3.4% at 1200 ng/ml and 7.3% at 20 ng/ml. Between-day coefficients of variation were no greater than 16%. Phenytoin and ACC-9653 did not interfere with the diazepam assay.

The area under the plasma concentration–time curve (AUC) for diazepam and phenytoin was determined by the trapezoidal rule and extrapolated to infinity by adding the ratio of the last observed concentration and the terminal elimination rate constant. The volume of distribution at steady state (V_{dss}) for diazepam was determined by the equation

$$V_{dss} = (\text{dose} * \text{AUMC}/\text{AUC}^2) - T(\text{dose})/(2 * \text{AUC})$$

where AUMC is the area under the moment curve and T is the time of infusion. Clearance (Cl) was determined by the ratio of dose to AUC. All parameters are reported as mean ± standard deviation.

Phenytoin peak concentrations (C_{max}) and the time of the maximum concentration (T_{max}) were determined by inspection of the concentration–time data. The conversion rate of ACC-9653 to phenytoin was determined by fitting a two-compartment iv infusion model to the individual ACC-9653 concentration–time data using PCNONLIN (Statistical Consultants, Inc., Lexington, KY). The rate constant, K_{10}, was considered to represent the conversion of ACC-9653 to phenytoin.

All statistical analyses were performed utilizing PC SAS (SAS Institute, Cary, NC). Within each treatment, the fraction unbound at each time point sampled was compared to the fraction unbound at time zero (before any drug was administered) by a paired t test (alpha = 0.005). The difference between the observed fraction unbound at each time point after drug administration and at time zero between treatments was tested by ANOVA (alpha = 0.005). The correlation coefficient (Pearson) of the fraction unbound for both ACC-9653 and phenytoin and the ACC-9653 plasma concentration was determined for both treatments. Statistical analyses for diazepam pharmacokinetic parameters were performed by ANOVA (alpha = 0.05). A paired t test was utilized to compare the AUC, C_{max}, and T_{max} for phenytoin as well as the difference between the mean concentration at each time point with regard to treatment (alpha = 0.005). A paired t test was also utilized to compare the conversion rate of ACC-9653 to phenytoin with regard to treatment.

RESULTS

Nine subjects were included in the study, ranging in age from 20 to 31 years; total body weight ranged from 69 to 99.4 kg. Pre- and post-study albumin values did not vary significantly. All albumin values were within the normal range (3.5–5.5 g/dl).

The diazepam fraction unbound ranged from 0.78 to 3.22% throughout the sampling period. Peak free diazepam concentrations (fraction unbound * observed C_{max}) ranged from 3.2 to 25.5 ng/ml. No differences were observed between treatments.

The fraction unbound of both phenytoin and ACC-9653 is presented in Fig. 1. Significant increases in the fraction unbound of both phenytoin and ACC-9653 occurred, with a maximum increase twice the baseline value. The increases were observed during both treatment periods, however, the return to the baseline value was prolonged for both phenytoin and ACC-9653 when diazepam was concomitantly administered. The maximum increase in the fraction unbound

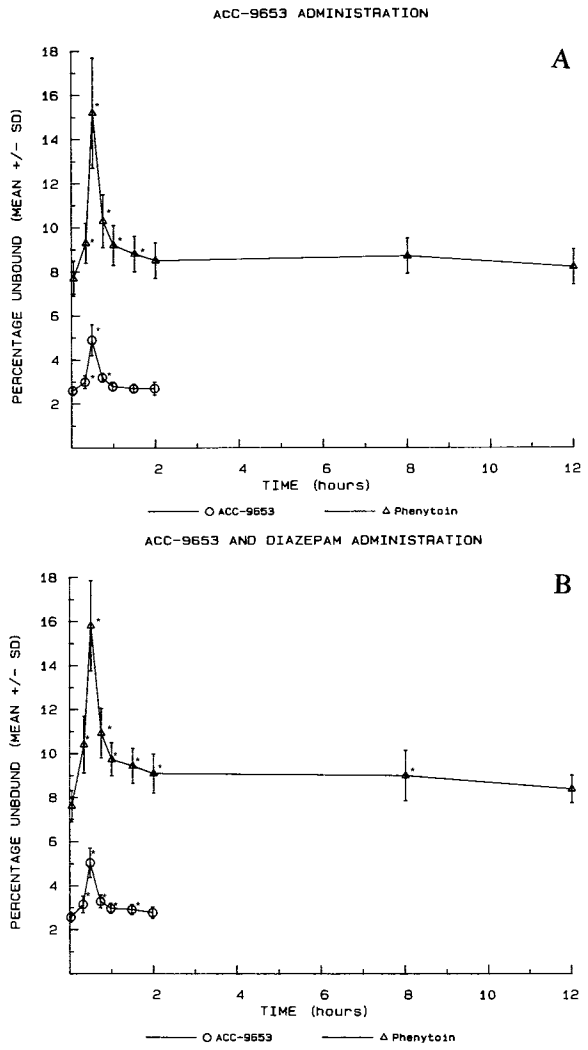

Fig. 1. Phenytoin (△) and ACC-9653 (○) percentage unbound (mean ± SD) vs time. The predose (time-zero) sample was drawn prior to the diazepam infusion; all other samples are plotted relative to the scheduled start time of the diazepam infusion. ACC-9653 was infused from 15 to 30 min. The phenytoin fraction unbound (expressed as percentage) was significantly greater [(*) $P < 0.005$] than the fraction unbound at time zero for 1 hr following the end of the ACC-9653 infusion when ACC-9653 was administered alone (A) and through the 8-hr time point when diazepam was coadministered (B). The ACC-9653 fraction unbound was significantly greater [(*) $P < 0.005$] than the fraction unbound at time zero for 15 min following the end of the ACC-9653 infusion when ACC-9653 was administered alone (A) and for 1 hr when diazepam was coadministered (B). In both treatments, the maximum fraction unbound of both phenytoin and ACC-9653 occurred at the end of the ACC-9653 infusion.

was observed at 30 min (end of the ACC-9653 infusion) in all cases, which was also the time of the maximum observed ACC-9653 plasma concentration. The concentration of ACC-9653 correlated significantly with the fraction unbound of both ACC-9653 and phenytoin ($r = 0.9$, $P = 0.0001$) in all cases.

Peak diazepam plasma concentrations ranged from 221.3 to 889 ng/ml. No significant differences in diazepam pharmacokinetic parameters or diazepam concentrations at any time point between treatments were observed. The mean AUC was 2524 ± 1678 ng * hr/ml when ACC-9653 was concomitantly administered (treatment B) and 2934 ± 2282 ng * hr/ml when diazepam was administered alone (treatment C). The mean clearance was 5.2 ± 2.5 liters/hr after treatment B and 5.3 ± 3.4 liters/hr after treatment C, and the mean V_{dss} was 13.0 ± 3.6 liters/kg following treatment B and 15.7 ± 5.9 liters/kg following treatment C.

Phenytoin concentrations were comparable between treatments at all time points except at 20 min (5 min into the ACC-9653 infusion). The mean concentration at 20 min during treatment A (ACC-9653 alone) was 0.66 ± 0.32 μg/ml, compared to 1.01 ± 0.42 μg/ml ($P = 0.0039$) during treatment B when diazepam was also administered (Table I). The AUC, C_{max}, and T_{max} for phenytoin did not differ significantly between treatments. The mean AUC was 426.6 ± 92.6 μg * hr/ml following treatment A and 417.4 ± 95.1 μg * hr/ml following treatment B. The mean C_{max} was 16.4 ± 2.4 μg/ml following treatment A and 15.4 ± 2.8 μg/ml following treatment B. The mean T_{max} occurred at 56.1 ± 16.7 min after treatment A and at 70.8 ± 37.2 min after treatment B.

ACC-9653 plasma concentrations also were comparable between treatments at all time points except at 20 min (5 min into the ACC-9653 infusion). The mean concentration at 20 min during treatment A was 41.92 ± 11.58 μg/ml, compared to 66.19 ± 7.66 μg/ml ($P = 0.0001$) during treatment B (Table I). The conversion rate half-life of ACC-9653 to phenytoin was significantly longer ($P = 0.0002$) when ACC-9653 was administered alone than when diazepam was concurrently administered (12.90 ± 3.06 min during treatment A vs. 9.53 ± 1.93 min during treatment B).

Drowsiness was the most commonly reported adverse event, the duration of which was considerably longer (1–4 hr) when both diazepam and ACC-9653 were administered than when diazepam was administered alone. Itching and tingling over the upper body, lower back, and groin were noted in five subjects during the ACC-9653 infusion (two of which experienced this event during both ACC-9653 treatment periods).

During the ACC-9653 infusion, itching at the site of administration was reported by two subjects. During the 72-hr sampling period following the drug infusion, tenderness in four subjects and erythema in three subjects were also noted. In one subject, the iv dose infiltrated during the ACC-9653 infusion (approximately 600 mg was infused); minimal swelling was noted for 2 hr but no complaints were reported after that time. No other complaints of irritation at the infusion site were associated with ACC-9653 administration.

Table I. Phenytoin and ACC-9653 Concentrations[a]

Time (min)	Phenytoin plasma concentration (μg/ml)		ACC-9653 plasma concentration (μg/ml)	
	Tx A	Tx B	Tx A	Tx B
20*	0.66 ± 0.32	1.01 ± 0.42	41.92 ± 11.58	66.19 ± 7.66
30	7.89 ± 3.27	8.38 ± 2.99	134.58 ± 20.00	145.87 ± 12.95
35	12.70 ± 3.96	13.33 ± 4.55	101.26 ± 22.40	102.37 ± 20.30
40	13.38 ± 4.47	14.05 ± 4.20	67.61 ± 19.80	64.17 ± 14.23
45	14.74 ± 3.19	13.73 ± 2.92	47.64 ± 14.76	46.88 ± 11.68
50	14.55 ± 2.30	13.54 ± 2.64	35.53 ± 10.89	33.74 ± 9.49
55	14.72 ± 2.22	10.95 ± 4.36	26.90 ± 9.39	25.14 ± 7.45
60	14.08 ± 1.47	12.98 ± 1.80	19.72 ± 7.12	21.06 ± 6.64
75	13.97 ± 1.45	12.95 ± 1.79	9.50 ± 4.41	9.28 ± 4.01
90	13.61 ± 1.60	12.53 ± 1.03	4.60 ± 2.24	5.05 ± 2.02
105	12.80 ± 1.66	12.06 ± 1.65	2.89 ± 1.71	2.43 ± 1.19
120	12.41 ± 1.48	12.34 ± 1.13	2.06 ± 0.93	1.59 ± 0.71
240	11.49 ± 1.01	11.11 ± 0.85	—	—
480	10.10 ± 1.37	9.59 ± 0.85	—	—
720	8.82 ± 1.11	8.69 ± 1.03	—	—
1440	6.95 ± 1.14	6.97 ± 1.01	—	—
2880	3.12 ± 0.97	2.99 ± 1.11	—	—
4320	1.08 ± 0.65	1.03 ± 0.66	—	—

[a] Time is relative to the scheduled time of the diazepam infusion, which was administered from 0 to 5 min. ACC-9653 was administered from 15 to 30 min. ACC-9653 was administered alone in treatment (Tx) A and was administered with diazepam in treatment B.

* The plasma concentrations were statistically different between treatments for both phenytoin ($P < 0.005$) and ACC-9653 ($P = 0.0001$) at 20 min (5 min after the start of the ACC-9653 infusion).

DISCUSSION

The pharmacokinetics of diazepam have been extensively reviewed (17). A two-compartment model, consisting of a rapid distribution (alpha), followed by a longer elimination (beta) phase is usually employed to describe the concentration–time profile of diazepam. A peak diazepam total concentration of 0.5 μg/ml has been associated with diazepam's anticonvulsant effect (18).

Diazepam is highly bound to plasma proteins. Previously reported values range from 96 to 98.6% (19–21). Protein binding of diazepam appears to be linear over the therapeutic range (19). Despite the fact that diazepam is highly protein bound, it has not been shown to displace other highly bound drugs, although valproic acid has been shown to displace diazepam from plasma protein binding sites (11,22,23). As demonstrated in this study, diazepam has no significant effect on the protein binding of phenytoin or ACC-9653. No effect on diazepam pharmacokinetic parameters (AUC, V_{dss}, Cl) was observed when ACC-9653 was coadministered.

The effect of ACC-9653 administration on the protein binding of phenytoin most likely results from displacement of phenytoin by ACC-9653. This displacement appears to be concentration dependent. These results also imply that the protein binding of ACC-9653 is concentration dependent. The increased fraction unbound of phenytoin may result in enhanced efficacy or increased toxicity, although the transient nature of the change in fraction unbound may minimize the clinical significance of this observation. Concomitant administration of diazepam delayed the return to baseline values of the fraction unbound of phenytoin and ACC-9653. The

mechanism of this specific interaction is unclear but is unlikely to be of clinical significance.

CONCLUSIONS

The lack of a significant pharmacokinetic drug interaction between ACC-9653 and diazepam suggests that these drugs may be safely administered together, although the results of this study should be confirmed in the intended patient population. The increase in phenytoin fraction unbound may be of clinical significance when ACC-9653 is administered.

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