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Report

Time-Dependent Absorption of Phenprobamate Following Multiple Dosing in Rats

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Unusual serum profiles of phenprobamate, a centrally skeletal muscle relaxant, were observed in Sprague Dawley rats receiving multiple doses of phenprobamate suspension. The concentrations of phenprobamate were higher after the morning doses than after the evening doses, synchronizing with the day–night pattern of drug administration. Crossover studies were conducted to investigate the apparent time-dependent kinetics of phenprobamate. Phenprobamate emulsion was orally administered as a single dose to a group of six rats at 0900 hr and again, after a washout period of 3 days, at 2100 hr. Another group of six rats was treated similarly with intraperitoneal drug administration. Blood samples were collected at various times for 12 hr. The AUCs were 146.56 ± 31.77 μg · hr/ml for the morning oral dose and 111.31 ± 21.32 μg · hr/ml for the evening oral dose ($P < 0.001$). Administered intraperitoneally, the AUCs were 179.89 ± 37.50 and 185.58 ± 28.51 μg · hr/ml for the morning and evening doses, respectively, the difference of which was not significant. The paired *t* test indicated a significant morning–evening difference in AUC following oral but not intraperitoneal drug administration. This suggests the absorption rather than metabolism as a contributing factor to the time-dependent kinetics of phenprobamate in rats.

KEY WORDS: phenprobamate; single dose; multiple dose; time-dependent kinetics.

INTRODUCTION

Circadian changes as a function of the time of drug administration have been demonstrated for a number of therapeutic agents. Indomethacin (1) and theophylline (2) exhibited plasma profiles with the highest peak concentration and shortest peak time when the drug was given in the morning, resembling the chronopharmacokinetics of ethanol (3). Contrary to ethanol, however, indomethacin and theophylline did not show a circadian rhythm for the area under the plasma concentration–time curve. Such differences in chronopharmacokinetics are conceivable as different circadian systems are involved in the absorption and/or disposition processes. Hollander *et al.* (4) studied the intestinal absorption of vitamin K at four different times (0600, 1200, 1800, and 2400 hr) in rats and found the rates of absorption in jejunum and ileum to be greater at midnight than at 0600 hr. They attributed the synchronization of absorption rate with time to the time of feeding rather than the pattern of illumination. A circadian rhythm was also described for the active transport of calcium in the rat intestine (5); the rhythm was synchronized to both the light–dark cycle and the time of food intake. Kabasakalian *et al.* (6) demonstrated in humans diurnal variations in the absorption of griseofulvin and found the peak urinary excretion rates at noon and the trough values in the morning. In general, the diurnal factors influencing drug absorption and disposition have not been adequately investigated.

Phenprobamate is a centrally acting skeletal muscle relaxant. Preliminary studies on the absorption, metabolism, and excretion of phenprobamate have been conducted in rats (7), rabbits (8), dogs (9), and humans (9). No studies to date have explored the pharmacokinetic chronicity of phenprobamate. During the time course of a multiple-dose kinetic study, we observed an apparent, time-synchronized appearance of serum concentrations, higher following daytime administrations and lower following evening-time administrations. The absorption and/or disposition factors could contribute to the time-dependent kinetic patterns of phenprobamate. To investigate further this apparent chronopharmacokinetics of phenprobamate, we administered the drug via two routes, as a single dose orally and intraperitoneally to rats in the morning and evening.

EXPERIMENTAL

Materials. Phenprobamate and acacia (Siegfried Ltd., Switzerland) were used as received. Benzoic acid, phosphoric acid, high-performance liquid chromatographic (HPLC)-grade methanol, and acetonitrile (Fisher Scientific

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Co., Pittsburg, Pa.) were used as supplied. Sodium clofibrate (ICI, England) was used as the internal standard for phenprobamate assay. Monobasic and dibasic phosphates (J. T. Baker Chemical Co., Phillipsburg, N.J.) were obtained as the sodium salts. Tween 20 and peanut oil (Sigma Chemical Co., St. Louis, Mo.) were used for the preparation of phenprobamate emulsion.

Phenprobamate Suspension. Phenprobamate suspension was prepared at a concentration of 20 mg/ml in acacia mucilage as described (10).

Phenprobamate Emulsion. An emulsion base of peanut oil, Tween 20, and ethanol (0.5:0.5:1.0) was used to emulsify phenprobamate to yield a concentration of 50 mg/ml.

Experimental Procedures. In the multiple-dose study, male Sprague Dawley rats were allowed free access to both food and water. Seven doses of phenprobamate suspension (100 mg/kg each) were administered to the rats by oral gavage at intervals of 4 hr (0900, 1300, 1700, 2100, 0100, 0500, and 0900 hr). Blood samples (0.2–0.3 ml) were collected from the tail vein just prior to each dose and for 12 hr following the last dose.

All rats used for the time-dependent studies were suspended from food but had ad libitum access to water 12 hr prior to drug administration. To investigate the time-dependent kinetics, phenprobamate emulsion (100 mg/kg) was orally administered to six rats at 0900 hr and again, after a washout period of 3 days, at 2100 hr. Blood samples (0.2–0.3 ml) were collected from the tail vein at 0, 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, and 12.0 hr. On a separate occasion, phenprobamate emulsion (100 mg/kg) was administered intraperitoneally to another group of six rats at 0900 hr and, after a washout period of 3 days, at 2100 hr.

Drug Analysis. Each serum sample was mixed with 100 μl of sodium clofibrate (2 μg) as the internal standard. After adding 400 μl of 1 *N* HCL, serum sample was extracted with 4 ml of ether. The ether extract was transferred to another tube and evaporated to dryness. The residue was reconstituted with 0.2 ml of methanol and an aliquot was subjected to HPLC analysis. Resolution of phenprobamate and clofibrate was achieved by using a mobile phase of 33% methanol, 15% acetonitrile, and 52% water (pH 2.5 adjusted by phosphoric acid) delivered at a flow rate of 2 ml/min through a C_{18} reverse-phase column. The UV absorption was monitored at 215 nm with attenuation of 0.01 AUFS (11).

Data Analysis. For the single-dose study, the area under the serum curve (AUC) was obtained by the trapezoidal method and extrapolated to infinite time. The peak concentration and peak time were observed from the serum concentration–time profile. For the multiple-dose study, the AUC was calculated over the entire dosing interval and extrapolated to infinite time following the last dose. For both studies, the elimination half-life was estimated by regression analysis of serum concentration data in the apparent log-linear phase.

Statistical Analysis. Between-group comparison of pharmacokinetic parameters was performed using the paired *t* test. A probability of $P < 0.05$ was considered statistically significant.

RESULTS

The serum profile of phenprobamate following multiple-dose administration is depicted in Fig. 1. Trough serum concentrations of phenprobamate were higher after the morning doses than after the evening doses. The area under the curve from zero to infinite was 275.77 ± 64.35 μg · hr/ml. The peak serum concentration of phenprobamate following the last (seventh) dose was 20.13 ± 6.92 μg/ml. The peak time was 1.50 ± 0.82 hr and the elimination half-life was 2.87 ± 0.71 hr.

Figure 2 depicts the serum profiles of phenprobamate following a single dose of oral emulsion in the morning and evening drug administrations. A comparison of pharmacokinetic parameters is presented in Table I. The AUCs exhibited a significant difference, 146.56 ± 31.77 μg · hr/ml for the morning dose and 111.31 ± 21.32 μg · hr/ml for the evening dose ($P < 0.001$). The elimination half-lives, however, were not significantly different, 2.95 ± 0.62 hr for the morning dose and 2.73 ± 0.37 hr for the evening dose.

Figure 3 depicts the serum profiles for phenprobamate administered to rats intraperitoneally in the morning and evening. The kinetic parameters of phenprobamate are presented in Table I. The areas under the curve were 179.89 ± 37.50 and 185.58 ± 28.51 μg · hr/ml for the morning and evening intraperitoneal administrations, respectively. The elimination half-lives were 2.15 ± 0.18 and 2.37 ± 0.22 hr, respectively, and were consistent with those obtained after oral administration. The relative bioavailabilities, po versus ip, were 81% for the morning dose and 60% for the evening dose.

DISCUSSION

An unusual serum profile of phenprobamate was observed following multiple doses of the drug over a 36-hr period. The terminal half-life following the last (seventh) dose was not significantly different from that following the single-dose study (11), suggesting that the elimination kinetics were probably not altered by multiple-dose drug administration. Therefore, a hypothesis was made that the time of drug administration affects the absorption of phenprobamate. To substantiate this hypothesis, phenprobamate was adminis-

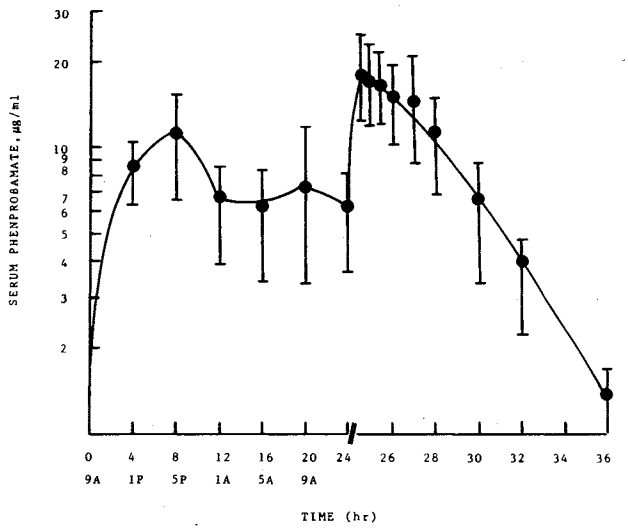

Fig. 1. Serum concentration versus time plot for phenprobamate in rats following seven oral doses (100 mg/kg each) of phenprobamate.

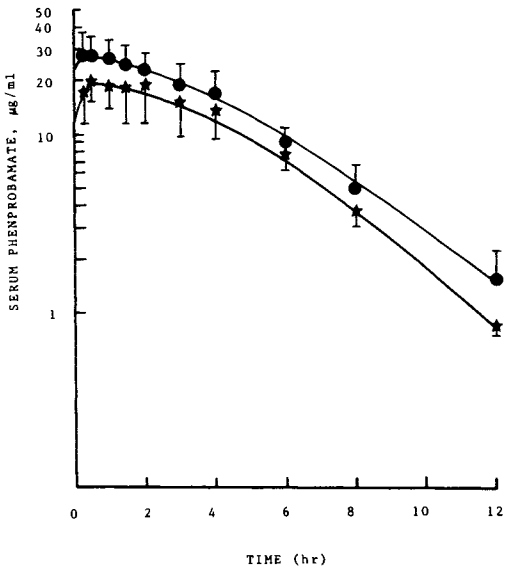

Fig. 2. Serum concentrations of phenprobamate in rats following oral administrations of a 100-mg/kg dose in the morning (——●——) and in the evening (——★——).

tered via the intraperitoneal and oral routes, and the kinetic parameters from the morning/evening doses were compared. Phenprobamate emulsion was used instead of suspension in order to determine the relative bioavailability from time-dependent study. Based on the intraperitoneal studies, the time of drug administration was not found to affect any of the pharmacokinetic parameters, including AUC and $t_{1/2}$. Intraperitoneal absorption of phenprobamate was very rapid, approximately an intravenous input. Thus, the lack of time effects on practically all parameters following intraperitoneal administration suggests that the elimination, and presumably metabolism, of phenprobamate was not influenced by the time of administration. On the other hand, the AUC was significantly different between morning and evening oral administrations, indicating that the absorption was indeed influenced by the time of drug administration. The consistent intraperitoneal and oral half-lives also suggested similar elimination kinetics regardless of the route and time of drug administration. Overall, the kinetic data contrasting the ip/po routes and morning/evening times of drug administration supported the time-dependent absorption of phenprobamate following multiple dosing.

Comparison of intraperitoneal and oral kinetics of phenprobamate indicated that the drug was absorbed more from the morning doses than the evening doses. The AUCs were 23 and 66% larger after ip than po administration in the morning and evening. Thus, this study also demonstrated the incomplete absorption of phenprobamate suspension following oral administration. When the oral absorption of emulsion was compared to suspension in rats (11), phenprobamate appeared to be absorbed to the same extent from both formulations. In humans, phenprobamate absorption from the commercial tablets was estimated at 80% (9).

Many factors could contribute to the time-dependent absorption of a drug administered via the oral route, among which are food intake, bile flow, and illumination. Schatz and Jahn (9) observed in humans a drop in phenprobamate blood concentration between 6 and 8 hr of a multiple-dose regimen and attributed this to the intake of lunch. Hollander *et al.* (4) also attributed the time-synchronized absorption of vitamin K in rats to the time of feeding. In our multiple-dose study, rats were allowed free access to food and water at all times except at the times of drug administration and blood collection. It is well recognized that rodents consume food primarily during the darkness cycle of the night time. Thus, it can be argued that the time of food consumption may have contributed to the concentration drop in the night time, as observed in our multiple-dose study. However, our study on feeding effect (13) suggested that food affected only the rate, and not the extent, of phenprobamate absorption, and also, rats used for this time-dependent study were fasted. Therefore the food effect on circadian absorption of phenprobamate was excluded. Wober and Nagel (5) reported the active transport of calcium in rat intestine as a function of both the light/darkness cycle and the time of food intake. In our multiple-dose phenprobamate study, the illumination was controlled at a normal cycle, light for 0900/2100 hr and darkness for 2100/0900 hr. Thus, we are not able to exclude the illumination effect on drug absorption if it indeed is a contributing factor. To elucidate the effect of illumination on drug absorption, a reversed light/darkness cycle will be implemented in the future study design.

Some research groups have demonstrated the circadian distribution of bile acids and its effects on drug absorption in rats (14,15) and hamsters (16). In most rodents, the bile salt content in the lumen of the proximal small intestine increased from the nadir at 4 PM to the peak at midnight. Thus, the rhythmic distribution of bile salt would presumably enhance the absorption of phenprobamate in the case of nighttime drug administration. On the contrary, our study showed greater absorption of phenprobamate following daytime ad-

Table I. Comparison of Pharmacokinetic Parameters of Phenprobamate in Rats for Oral and Intraperitoneal Administrations in the Morning and Evening (Mean ± SD; $N = 6$)

	Oral dose		Intraperitoneal dose	
	AUC (μg · hr/ml)	$t_{1/2}$ (hr)	AUC (μg · hr/ml)	$t_{1/2}$ (hr)
Morning	146.56 ± 31.77	2.95 ± 0.62	179.89 ± 37.50	2.15 ± 0.18
Evening	111.31 ± 21.32	2.73 ± 0.37	185.58 ± 28.51	2.37 ± 0.22
t test	7.00	0.91	0.33	1.55
Significance	$P < 0.001$	NS[a]	NS	NS

[a] Not significant.

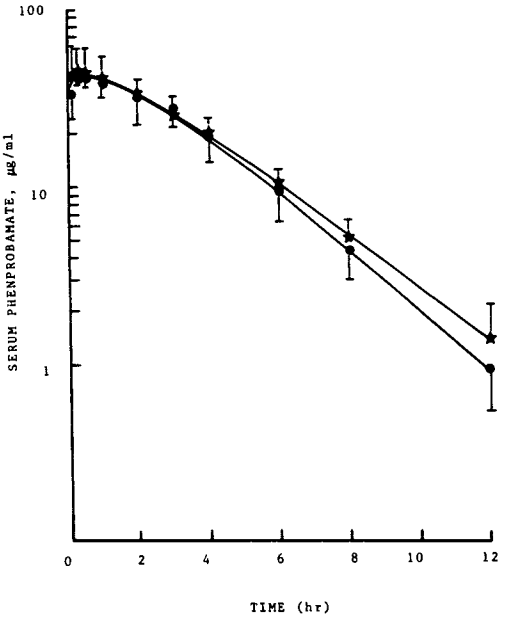

Fig. 3. Serum concentrations of phenprobamate in rats following intraperitoneal administrations of a 100-mg/kg dose in the morning (—●—) and in the evening (—★—).

ministration. Therefore, the bile salt distribution pattern did not seem to contribute to the decline of the nighttime serum concentration following multiple-dose administration of phenprobamate.

Phenprobamate is a lipid-soluble compound and its absorption is presumably rate limited by the rate of blood perfusion in the gastrointestinal (GI) tract. Any neurohumoral alteration in the gastrointestinal tract as a change of circadian rhythm may affect drug absorption. Thus further investigation will focus on *in situ* GI model to study the time-dependent absorption. Meanwhile the GI blood flow will be determined in order to demonstrate the relationship between absorption of the drug and blood flow.

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