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Suitability of the dog as an animal model for evaluating theophylline absorption and food effects from different formulations

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Summary

This study evaluates the absorption of theophylline in humans and dogs from formulations exhibiting differences in in vitro dissolution profiles under fasting conditions. In addition, food-induced changes in the kinetics of absorption of theophylline from different formulations were examined. After administration of three experimental controlled-release (CR) formulations under fasting conditions, the directions of changes in the rate and extent of theophylline absorption found in the dog were similar to those determined in humans. Furthermore, food-induced changes in the rate and extent of absorption found in humans were confirmed in the dog. It is concluded that the dog is a suitable animal model for assessing the absorption characteristics and food effects with different formulations of theophylline.

Introduction

Theophylline has long been used in the treatment of asthma. The therapeutic serum/plasma concentrations of theophylline associated with optimal likelihood of benefit and minimal risk of toxicity are considered to be between 8 and 20 μg/ml (Mitenko and Ogilvie, 1973; Pallock et al., 1977).

The elimination half-life of theophylline, influenced by numerous factors, lies within the ranges 3–5 h in most children and 5–9 h among nonsmoking healthy subjects. Therefore, a frequent dosing schedule (every 6–8 h) is required when an immediate-release formulation is used. Even with this frequent dosing schedule, serum/plasma concentrations of theophylline tend to vary beyond the therapeutic range (Hendeles et al., 1980). Controlled-release (CR) formulations of theophylline are widely used to maintain a steady-state theophylline serum/plasma concentration of 8–20 μg/ml with less frequent dosing (Hendeles et al., 1980).

Recently, it has been reported that the absorption of theophylline from CR formulations can be markedly affected when administered with a high fatty meal (Hendeles et al., 1985a,b; Karim et al.,

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1985, 1986). The absorption changes include: (a) increase in rate; (b) increase in extent; (c) decrease in extent; (d) increase in rate and extent; and (e) more variable absorption. The objective of the present study was to determine whether the dog was a suitable animal model for reliable prediction of the absorption characteristics of theophylline and the direction of food-induced absorption changes in humans after administration of theophylline CR formulations.

Materials and Methods

Formulations

Three experimental CR formulations (lot nos. RCT 7244, 7239 and 7241 designated formulations A–C, respectively) containing 300 mg theophylline per capsule were prepared in order to provide different rates of theophylline dissolution in vitro (Fig. 1) and administered to humans under fasting conditions. In addition, 200-mg capsules containing the same three lots of theophylline beads were prepared and administered to dogs in fasting states. The formulations used in the study under non-fasting states were a production lot (lot no. 1283-873) of Theo-24 300-mg capsules (Searle) for humans, 200-mg capsules of the same production lot of Theo-24 beads for dogs and commercially purchased Slo-Bid (200 mg), theophylline anhydrous USP tablets (200 mg) and aminophylline tablets (200 mg) for dogs.

Clinical studies

Formulation effect. Absorption of theophylline from the five different CR formulations (three Searle experimental CR formulations A–C and two other formulations not reported here) were examined at steady state in 30 healthy male nonsmokers (age 19–37 years, weight 60.5–90.9 kg) under fasting conditions. Each of 30 subjects received three out of five formulations according to a randomized, balanced incomplete block design (Cochran and Cox, 1957; Gill, 1978). For each formulation, 18 of the 30 subjects were tested. Drug dosing of 900 mg theophylline (three 300-mg capsules with 240 ml water) per day was done for five days with a washout period of seven days after each treatment. In addition, the same subjects received a 600 mg single dose of aminophylline immediate-release tablets. Subjects fasted for at least 10 h before dosing in the morning and for an additional 2 h after dosing. Standardized meals were taken at 9:00 a.m., 1:30 p.m. and 6:30 p.m., with a snack at 9:00 p.m. Subjects were confined to the study unit for the entire duration of the study. Ingestion of alcohol or any xanthine-containing food or beverages (e.g., chocolate, coffee, tea and colas) was forbidden. Blood samples were collected prior to each dose administration to determine the attainment of a steady state and at specified time intervals over a 24 h time period on day 5.

Food effect. Effect of food on absorption was assessed in a single-dose study in 20 subjects. The subjects received three 300-mg Searle CR capsules (lot no. 1283-873) under fasting and non-fasting (Karim, 1986) conditions (two treatments) and three other treatments in a randomized, crossover design. Prior to and after the food effect study, the same 20 subjects received 400 mg aminophylline (two 200-mg tablets, lot no. 1180–344) which was equivalent to 480 mg theophylline under fasting conditions. During the fasting treatment periods, the subjects were fasted overnight for 10 h, received the drug at 8:00 a.m. and received the standardized breakfast 4 h later at 12:00. During the non-fasting treatment period, the subjects fasted overnight for 10 h received the standardized breakfast at 7:45 a.m. and the drug at 8:00 a.m. The standardized high-fat breakfast consisted of the following: two slices of toasted white bread spread with butter, two eggs fried in butter, two slices of bacon, 2 ounces of hash browned potatoes and 8 ounces of whole milk. The nutritional content of the meal (870 calories) was approx. 33 g protein, 58 g fat, 58 g carbohydrate. Blood samples were taken at specified time intervals over a 72 h period.

Animal studies

Formulation effect. A study of theophylline absorption was conducted in six healthy female beagle dogs (age 1–5 years, weight 8.0–12.5 kg) which received each of the three Searle CR formulations A–C under fasting conditions. The animals

received in a randomized, crossover manner, 200 mg of theophylline (one 200 mg capsule) once a day for 4 days. The CR formulations were prepared with the same theophylline beads as used in the human study. The washout period between each treatment was 10 days. Blood samples were taken at specified time intervals over a 24 h period on day 4–5. Additional blood samples were collected prior to each dose administration to assess steady state achievement of plasma concentrations.

Food effect. Food-induced absorption changes were examined with Searle Theo-24 CR (200 mg capsule) and three additional commercially available formulations (Slo-Bid, aminophylline tablet and theophylline anhydrous USP tablet). For each formulation, each of the six dogs received 200 mg of theophylline under fasting and non-fasting conditions in a two-way randomized crossover study. In the non-fasting states, food for the dog was prepared by homogenizing the same standardized high-fat meal as that given to humans. The food was given 15 min prior to dosing. Blood samples were taken at specified time intervals over a 24 h period.

In vitro dissolution methods

The in vitro dissolution of theophylline from all formulations was determined in USPXX apparatus I (baskets) at 37 °C at a stirring rate of 100 rpm. The dissolution media (1000 ml) was 0.05 M phosphate buffer at pH 6.6.

Analytical method

Serum concentrations of theophylline were determined by an enzyme immunoassay system (EMIT^R, Syva, Palo Alto, CA). The assay sensitivity was 0.6 μg/ml.

Statistical and pharmacokinetic analysis

For the formulation effect study in humans, univariate analyses of variance were performed to compare differences in peak serum concentrations (C_{max}), time to reach C_{max} (T_{max}) and area under the plasma concentration-time curve (AUC) from 0 to 24 h among the three CR formulations following repetitive drug administration. The model used to compare the formulations consisted of terms for sequence of formulations, subject, formulation and study period. Least-squares means were computed for each formulation and compared using Tukey's multiple comparison procedure (Neter and Wasserman, 1974). Similarly, for studying the formulation effect in the dog, univariate analyses of variance were performed on the same pharmacokinetic parameters. The model used to compare the formulations consisted of terms for animal, formulation and study period. Least-squares means were compared using Tukey's multiple comparison procedure.

In the food-induced absorption study in humans, the pharmacokinetic parameters with and without food were compared using multivariate analysis of variance and *t*-tests. In dogs the pharmacokinetic parameters with and without food were compared using univariate analysis of variance and *t*-tests. In all cases, the *t*-tests were performed using a two-tailed hypothesis.

The fraction of dose absorbed as a function of time was calculated according to the method of Wagner and Nelson (1964) after a single dose and the modified Wagner-Nelson equation (Wagner, 1983) after multidose administration. The fraction of dose absorbed was calculated assuming that theophylline absorption after oral administration of aminophylline immediate release formulation was 100%. In this calculation, linear regression estimates of the slopes of the terminal phase portion of the log concentration-time curves (last four to five time points) after aminophylline administration were used as the elimination rate constants.

Results

In vitro dissolution

Fig. 1 shows the mean ($N = 12$) in vitro dissolution profiles of the three experimental Searle CR formulations (A–C) determined in pH 6.6 phosphate buffer at 37 °C and with a stirrer speed of 100 rpm. The mean dissolution rates were in decreasing order for formulations A–C. The mean values of the dose dissolved in 8 h were 83.5, 66.9 and 55.0% for formulations A–C, respectively.

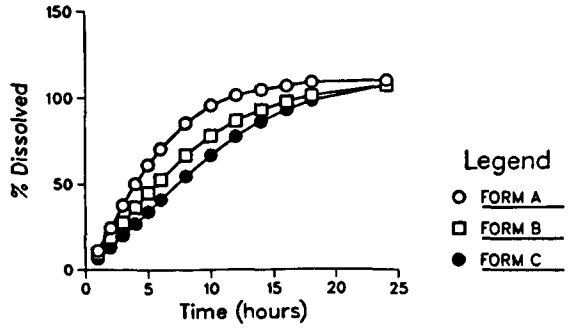

Fig. 1. Mean ($N = 12$) in vitro dissolution profiles of theophylline from three CR formulations in pH 6.6 phosphate buffer at 37 °C with a stirrer rate of 100 rpm.

Formulation effect

Fig. 2 shows mean plasma concentrations of theophylline following once-a-day multiple doses of CR formulations A–C to dogs and humans under fasting conditions. The mean pharmacokinetic parameters are summarized in Tables 1 and 2.

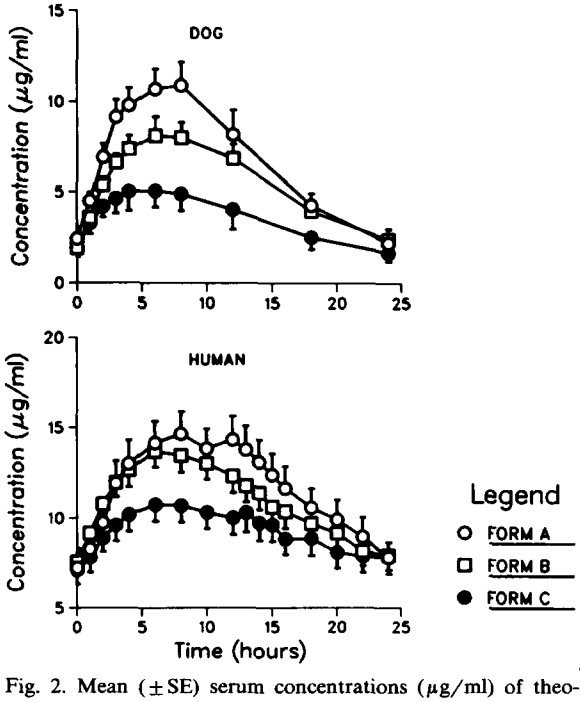

Fig. 2. Mean (±SE) serum concentrations (μg/ml) of theophylline after repetitive daily single-dose administration of three Searle CR formulations to six beagle dogs (200 mg/animal) and 18 subjects (900 mg/subject) under fasting conditions.

TABLE 1

Mean (± SE) pharmacokinetic parameters of theophylline in the dog on day 4 following repetitive oral administration of the three CR formulations under fasting conditions at a single daily dose of 200 mg/dog

Pharmacokinetic parameters	Formulation		
	A ($N = 6$)	B ($N = 6$)	C ($N = 6$)
C_{max} (μg ml^{-1})	11.4 ± 1.2	8.62 ± 0.91	5.60 ± 0.93
C_{min} (μg ml^{-1})	2.22 ± 0.36	2.42 ± 0.58	1.67 ± 0.47
T_{max} (h)	5.8 ± 0.8	7.3 ± 1.1	5.2 ± 1.1
AUC (μg h ml^{-1})	164 ± 18	133 ± 12	85.9 ± 17.1
F relative (%) [a]	63.8 ± 10.6	48.8 ± 3.1	22.3 ± 3.3

[a] Systemic availability relative to aminophylline immediate release formulation.

In the dog, the rate and extent of theophylline absorption were in decreasing order of formulations A–C, as expected from in vitro dissolution data (Fig. 3). The means (±SE) of the dose absorbed in the dog in 24 h were 63.8 ± 10.6, 48.8 ± 3.1 and 22.3 ± 3.3% for formulations A–C, respectively. The steady-state C_{max} values in the dog were significantly different ($p < 0.05$) between formulation A and C. However, no significant difference was observed between formulation B and either A or C (Table 3). The steady-state AUC values showed significant differences between formulation C and either A or B while none occurred between formulations A and B. The mean

TABLE 2

Mean (± SE) pharmacokinetic parameters of theophylline in healthy subjects on day 5 following repetitive oral administration of the three CR formulations under fasting conditions at a single daily dose of 900 mg/man

Pharmacokinetic parameters	Formulation		
	A ($N = 18$)	B ($N = 18$)	C ($N = 18$)
C_{max} (μg ml^{-1})	15.9 ± 1.4	14.3 ± 0.9	11.8 ± 0.9
C_{min} (μg ml^{-1})	6.68 ± 0.93	6.49 ± 0.68	6.17 ± 0.78
T_{max} (h)	9.0 ± 0.7	8.3 ± 1.1	8.1 ± 1.0
AUC 0 ± 24 h (μg h ml^{-1})	283 ± 27	265 ± 20	224 ± 21
F relative [a] (%)	100.0 ± 5.8	95.0 ± 6.6	87.1 ± 7.5

[a] Systemic availability relative to aminophylline immediate release formulation.

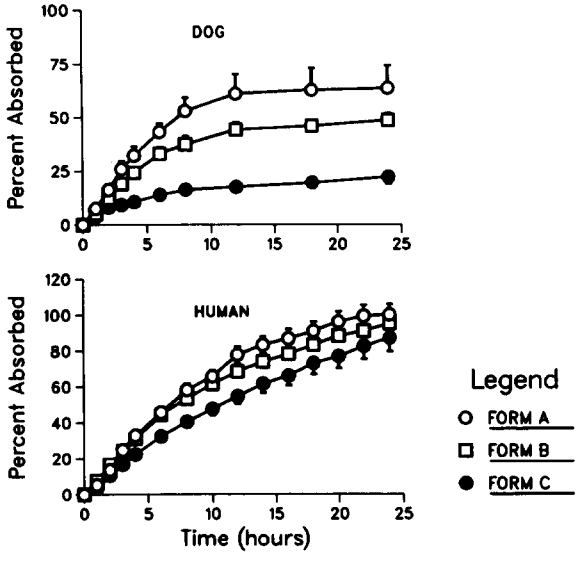

Fig. 3. Mean (±SE) percentages of the dose absorbed after repetitive daily single-dose administration of three Searle CR formulations to six beagle dogs (200 mg/animal) and 18 subjects (900 mg/subject) under fasting conditions.

TABLE 3

Least square mean differences of C_{max} and AUC comparing three Searle theophylline CR formulations in dogs and humans

Formulation comparison	C_{max} (μg/ml)		AUC (μg h ml^{-1})	
	Dog	Man	Dog	Man
A vs B	2.77	2.4	31.0	42.2 *
A vs C	5.78 *	3.8 *	78.0 *	53.9 *
B vs C	3.02	1.4	47.0 *	11.7

* Significant at $\alpha = 0.05$.

times to reach C_{max} (T_{max}) were not significantly ($p > 0.05$) different amongst the three formulations A–C.

In humans, the rate and extent of theophylline absorption after repetitive dose administration under fasting conditions were also in decreasing order of formulations A–C (Fig. 3). However, the extent of absorption was greater in humans than in dogs for all formulations. Theophylline was continuously absorbed in humans and the mean values of

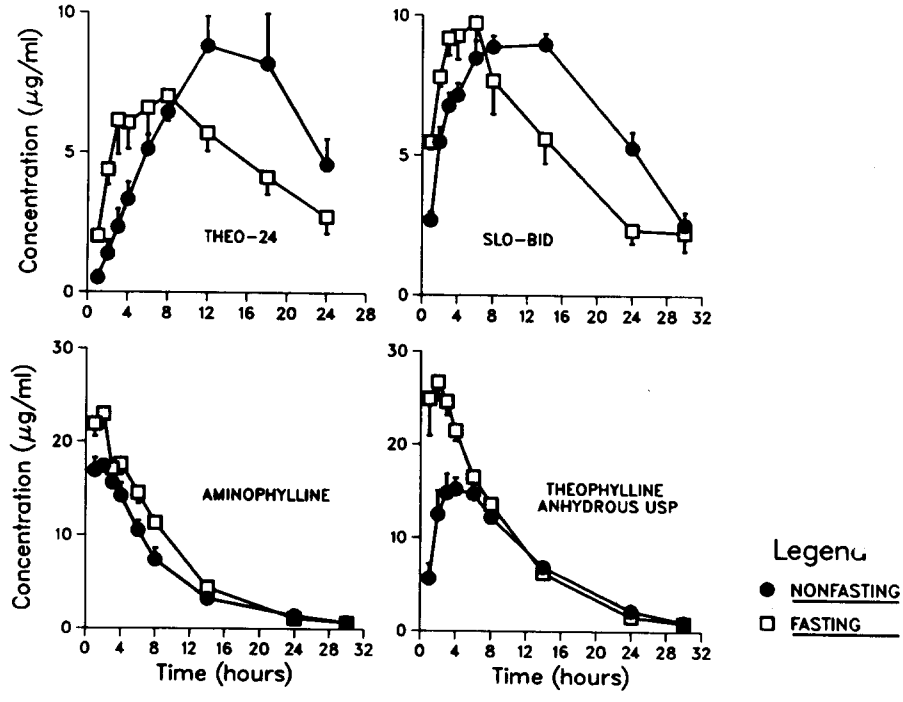

Fig. 4. Mean (±SE) serum concentrations of theophylline after a single oral dose (200 mg/animal) administration of the drug as four different formulations to six beagle dogs under fasting conditions and immediately after a high-fat breakfast.

TABLE 4

Mean (± SE) pharmacokinetic parameters of theophylline after single oral administration of four different formulations to dogs (200 mg / animal) under fasting conditions and immediately after high-fat breakfast

Formulation	Food or Fast	C_{max} ($\mu g\ ml^{-1}$)	T_{max} (h)	AUC 0–24 h ($\mu g\ h\ ml^{-1}$)
Searle Theo-24				
	Fast	7.33 ± 1.06	6.5 ±1.0	117 ± 17
($N = 6$)	Food	9.85 ± 1.49	12.3 ±1.3	146 ± 24
Statistical significance		*	*	N.S.
Slo-Bid	Fast	9.95 ± 0.94	5.50 ± 0.5	153 ± 15
($N = 6$)	Food	9.42 ± 0.44	12.7 ±1.5	176 ± 8
Statistical significance		N.S.	*	N.S.
Aminophylline	Fast	23.3 ±1.6	1.7 ±0.2	203 ± 11
($N = 6$)	Food	18.2 ±0.8	1.3 ±0.2	154 ± 13
Statistical significance		*	N.S.	*
Theophylline Anhydrous USP	Fast	28.1 ±2.5	1.7 ±0.3	254 ± 21
($N = 6$)	Food	17.4 ±1.4	4.7 ±0.8	200 ± 11
Statistical significance		*	*	*

* Difference in the pharmacokinetic parameter with and without food is significant ($p < 0.05$). N.S., difference is not significant ($p > 0.05$).

the dose absorbed in 24 h were 100 ± 6, 95.0 ± 6.6 and 87.1 ± 7.5% for formulations A–C, respectively (Table 2).

As in dogs, the steady-state C_{max} values in humans were significantly different only between formulations A and C (Table 3). The steady-state AUC values in humans were significantly different between formulation A and either B or C. T_{max} values in humans were not significantly different among the three formulations as observed in dogs.

Food effect

Fig. 4 shows single-dose theophylline serum concentration-time curves for four formulations given to six dogs under fasting and non-fasting conditions. The mean pharmacokinetic parameters are summarized in Table 4. With reference to the fasting state, there were increases in the mean C_{max} (34%, $p < 0.05$) and AUC (25%, $p > 0.05$) values with Theo-24 (Searle) although the observed increase in AUC was not statistically significant. The absorption rate under fasting conditions was greater up to 8 h but slower thereafter, as compared to non-fasting conditions (Fig. 5, upper panel). Consequently, a significant delay occurred in T_{max} with food.

Fig. 6 shows the mean serum concentrations of theophylline after the administration of a single oral dose of Theo-24 to healthy subjects under fasting conditions and immediately after high-fat breakfast. Mean C_{max} and AUC values increased by approx. 60% ($p < 0.05$) and 17%, respectively ($p < 0.05$) with food (Table 5). As observed in dogs, the absorption rate of theophylline in humans under fasting conditions was greater up to 6 h but slower thereafter as compared to non-fasting conditions (Fig. 5, lower panel). The observed mean T_{max} was slightly delayed with food but the difference in T_{max} under fasting and non-fasting conditions was not statistically significant.

For Slo-Bid capsules, absorption in the dog was significantly delayed ($p < 0.05$) with food but no significant differences were found with C_{max} and AUC values under fasting and non-fasting conditions (Table 4). With the immediate-release aminophylline and theophylline anhydrous USP formulations, C_{max} and AUC values in the dog decreased significantly ($p < 0.05$) with food. T_{max}

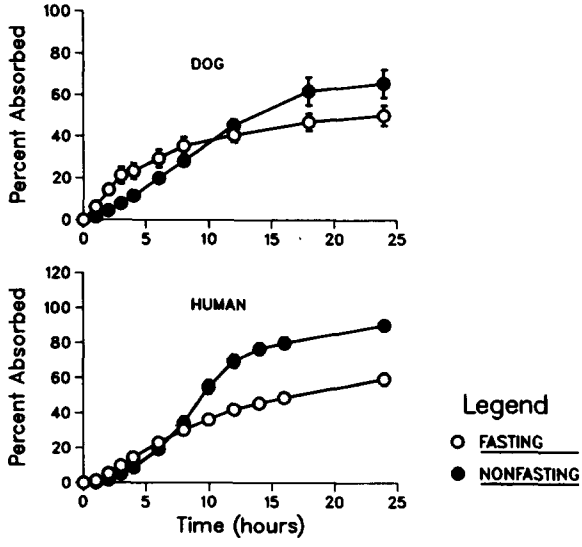

Fig. 5. Mean (±SE) percentages of the dose absorbed after single oral dose administration of the drug as Theo-24 formulations to six beagle dogs (200 mg/animal) and 20 subjects (900 mg/subject) under fasting conditions and immediately after a high-fat breakfast.

values were similar with and without food for aminophylline but significantly delayed with food for theophylline anhydrous USP.

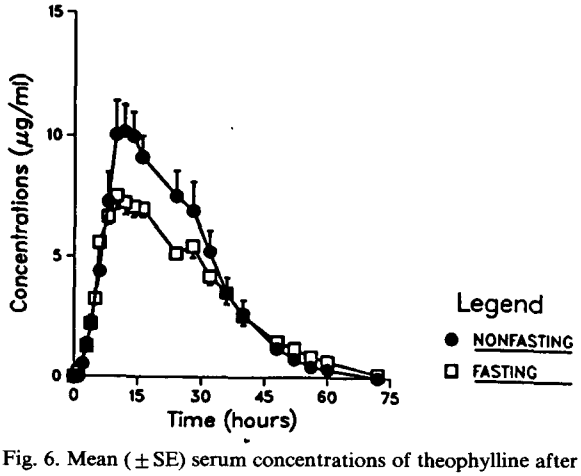

Fig. 6. Mean (±SE) serum concentrations of theophylline after a single oral dose (900 mg/subject) administration of the drug as Theo-24 formulation to 20 healthy subjects under fasting conditions and immediately after a high-fat breakfast.

Discussion

The dog has been widely used for bioavailability assessment of many drug preparations (Aoyagi et al., 1982; Ogata et al., 1982, 1984, 1985, 1986). Since the length of each part of the gastrointestinal tract, transit time and basal secretion are different in the dog and human, depending upon the drug substance, a substantial difference in the rate and/or extent of absorption is expected between these two species. However, it has been shown that the dog is a good animal model for predicting absorption of theophylline in humans under fasting conditions (Hussein et al., 1987).

The present study demonstrates that the rate and extent of theophylline absorption in female dogs after administration of the three Searle CR formulations decreased in the same order as in male humans under fasting conditions, which is in good agreement with the results reported by Hussein et al. (1987). In addition, our study shows that the dog is a good model to predict food-induced absorption changes with theophylline formulations in humans. Increases in the rate and extent of absorption in man reported for the Searle CR formulations were also reproduced in the dog which was dosed with the same formulations.

With Slo-Bid, a food induced decrease in the rate without changes in the extent of theophylline absorption in man has been previously observed (Weinberger et al., 1986). These food-induced absorption changes could also be reproduced in the dog.

TABLE 5

Mean (± SE) pharmacokinetic parameters of theophylline after single oral dose administration of Searle Theo-24 formulation (900 mg/subject) to healthy subjects with and without food

Food or Fast	C_{max} ($\mu g\ ml^{-1}$)	T_{max} (h^{-1})	AUC 0–72 h ($\mu g\ h\ ml^{-1}$)
Food ($N = 20$)	13.0±0.9	15.7±2.1	272±22
Fast ($N = 20$)	8.1±0.49	13.0±1.6	233± 1.5
Statistical significance	*	N.S.	*

* Difference in the pharmacokinetic parameters with and without food is significant ($p < 0.05$). N.S., difference is not significant ($p > 0.05$).

After administration of an immediate-release formulation Tedral [R] to humans, the mean C_{max} was reduced with food (Welling et al., 1975). Similar results were observed in the dog after administration of immediate-release formulations of aminophylline and theophylline anhydrous USP. Therefore, it can be concluded that the dog is a good model to predict food-induced absorption changes in man associated with different formulations of theophylline.

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