The Effect of Gastric pH on the Absorption of Controlled-Release Theophylline Dosage Forms in Humans

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The bioavailability of three marketed controlled-release dosage forms and a reference solution of theophylline was studied in eight subjects with normal gastric fluid acidity and seven subjects who were achlorhydric. Gastric pH was monitored with a Heidelberg capsule. One of the controlled-release dosage forms dissolved more rapidly *in vitro* when exposed to acid conditions, one dissolved more rapidly in pH 7.5 media, and the third dissolved at a rate independent of pH. Using a crossover design, each subject received each dosage form twice. Blood was sampled for up to 47 hr after each dose, and serum was assayed for theophylline by HPLC. The product which dissolved more rapidly under acid conditions *in vitro* exhibited a 3 hr longer T_{max} in the achlorhydrics compared to the normal subjects. The product which dissolved more rapidly in the pH 7.5 media exhibited a relatively higher AUC(0–∞) in the achlorhydric subjects than in normal subjects after the AUC data were normalized for clearance differences between the two subject groups. The *in vivo* bioavailability of these dosage forms could be related to the *in vitro* dissolution characteristics for some parameters. However, with the exception of the mean T_{max} values, the mean bioavailability parameters differed by less than 20% between the two subject groups.

KEY WORDS: controlled-release theophylline; pH-dependent dissolution; achlorhydric humans; bioavailability.

INTRODUCTION

In recent years there have been reports that some oral dosage forms can exhibit pH-dependent *in vitro* dissolution characteristics. Examples include diazepam tablets (1,2) and controlled-release formulations of quinidine gluconate (3), ibuprofen (4), and theophylline (5). It is also known that some individuals are achlorhydric, i.e., are unable to secrete gastric acid, due to aging or certain disease states (6,7). In addition, many individuals take medications which elevate gastric fluid pH. Thus, there is a concern that a dosage form with pH-dependent *in vitro* dissolution properties may exhibit altered absorption of drug from the gastrointestinal tract in these patients. Since most bioequivalence studies are conducted in healthy young male volunteers with presumably normal gastrointestinal physiology, it remains to be determined if certain patient populations may exhibit increased or decreased bioavailability as a function of the pH of their gastric fluid.

The objective of this investigation was to compare the bioavailability of three controlled-release theophylline dosage forms with different types of pH-dependent *in vitro* dissolution characteristics in humans with either normal gastric pH or achlorhydria.

MATERIALS AND METHODS

Experimental Design

The experimental design is given in Table I. Three controlled-release theophylline products and a reference solution were each to be given on two separate occasions to a group of eight subjects with achlorhydria and a group of eight normal subjects. Phase 1 was completed prior to starting Phase 2. At least 1 week elapsed between each dose.

The results of the *in vitro* dissolution testing of two of the three controlled-release products (S and N) have been previously reported (5). Briefly, Product S dissolved much more rapidly in USP simulated intestinal fluid than in USP simulated gastric fluid, using USP Apparatus I (Rotating Basket) at 100 rpm. Neither media contained enzymes. Product N was just the reverse, dissolving more rapidly in simulated gastric fluid, using USP Apparatus II (Paddle) at 50 rpm. However, when the dissolution of Products S and N in the simulated intestinal fluid was compared to the dissolution for 1 hr in simulated gastric fluid followed by 11 hr in simulated intestinal fluid, only Product N showed a notable change in dissolution, with about 50% greater dissolution in the mixed solvent system.

In the present study Products N and S were utilized, as well as a third controlled-release formulation (Product K) which did not exhibit pH-dependent dissolution. The differences in the percent of Product K dissolved in the simulated gastric fluid and simulated intestinal fluid did not exceed 6% using USP Apparatus II at 50 rpm.

Each study week the subjects were admitted to the University Clinical Research Center the evening prior to the day of dosing. They remained at the facility until the last blood sample was obtained. They were required to refrain from taking any type of drug from 1 week prior to the first study day until the end of the study. Alcohol ingestion was prohibited from 2 days prior to dosing until 2 days after each dose. All meals were standardized and were repeated for each of the eight study phases. No xanthine-containing food or beverages were allowed from 24 hr prior to each dose until the end of each blood collection period. The subjects were fasted for 10 hr prior to each dose and for 4 hr after each dose. A Heidelberg capsule was swallowed along with 60 mL of water approximately 1 hr prior to dosing and the pH of the gastric fluid was recorded. The Heidelberg capsule is a miniaturized pH electrode radiotransmitter which measures pH as previously described (4,8–13).

Each dose was administered with 180 mL of water. A 6-mL blood sample was obtained just prior to dosing and 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 15, 20, 25, 30, 35, and 47 hr after dosing each of the three controlled-release products. The 10-, 30-,

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Table I. Dosing Sequence for an Eight-Dose Study of Theophylline[a]

Subject no.					Treatment allocation							
Phase 1		Phase 2										
Gr 0	Gr 1	Gr 0	Gr 1	Sequence	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
1	2	9	10	1	L	N	K	S	S	K	N	L
3	4	11	12	2	K	L	S	N	N	S	L	K
5	6	13	14	3	S	K	N	L	L	N	K	S
7	8	15	16	4	N	S	L	K	K	L	S	N

[a] Odd-numbered subjects (Group 0) were documented to have achlorhydria; even-numbered subjects (Group 1) had a normal gastric pH. Subject 1 dropped out after Week 2. The letters within the matrix refer to the four test formulations: L—Somophylline-DF oral liquid, 300 mg anhydrous theophylline per 16.67 mL of solution (Fisons Corp., Bedford, MA); K—Theo-Dur, 300-mg controlled-release tablet (Key Pharmaceuticals, Miami, FL); S—Theo-24, 300-mg controlled-release capsule (Searle & Co., San Juan, PR); N—LaBID, 250-mg controlled-release tablet (Norwich Eaton, Norwich, NY).

and 47-hr samples were not obtained for the liquid product. The serum was harvested, and stored at −20°C until assayed.

Subject Selection

The identification of subjects with achlorhydria involved recruiting individuals with a medical history of pernicious anemia, a condition that is known to be accompanied by achlorhydria (14). A lack of gastric acid secretion was documented by aspiration of gastric fluid through a 14 French radiographic nasogastric tube during four consecutive 15-min time periods. A 6-μg/kg subcutaneous dose of pentagastrin (Ayerst Laboratories) was then administered to stimulate gastric fluid secretion, and the gastric fluid aspiration was repeated. The pH of the aspirated fluid was measured with a pH meter, the volume was measured, and an aliquot was titrated with sodium hydroxide to determine the milliequivalents of acid secreted. Prior to insertion of the nasogastric tube, the subjects also swallowed a Heidelberg capsule tethered with a suture line. Both the Heidelberg capsule and the nasogastric tube were positioned in the antrum of the stomach using fluoroscopy. The subjects were medically evaluated to insure they were in good health. The demographics of the subjects are summarized in Table II. A subject was considered to be achlorhydric if the gastric fluid pH was at least 6.0 with and without pentagastrin, and less than 2 mEq/hr of gastric acid was secreted after pentagastrin administration. A subject was considered normal if the gastric fluid pH was less than 3 and the pentagastrin stimulated gastric acid secretion was greater than 10 mEq/hr. The mean age was 51.3 and 44.6 years and the mean weight was 77.4 and 82.8 kg for the achlorhydrics and normals, respectively. The protocol was approved by the Institutional Review Board and all subjects were provided written informed consent.

In Vitro Studies of the Heidelberg Capsule

The stability of the pH measurement with the Heidel-

Table II. Subject Demographics[a]

Subject no.	Age (yr)	Sex	Weight (kg)	Race	Smoker	Cholecystectomy
1	58	F	90.9	C	No	Yes
2	55	F	80.5	C	No	Yes
3	42	F	67.7	B	Yes	No
4	54	F	64.5	B	Yes	No
5	68	M	90.9	C	Yes[b]	Yes
6	27	M	90.9	C	No	Yes
7	47	F	75.0	C	No	No
8	30	F	79.5	B	No	No
9	52	M	84.5	C	No	No
10	53	M	85.5	C	No	No
11	55	M	91.8	C	No	No
12	52	M	109.5	C	No	No
13	51	F	64.5	C	No	No
14	46	F	71.4	B	No	No
15	44	F	67.3	B	No	No
16	40	F	80.9	B	No	No

[a] Even-numbered subjects had a normal gastric pH; odd-numbered subjects were achlorhydric. Subject 1 withdrew from the study after week 2, and her data were not analyzed.

[b] Occasional pipe smoker.

berg capsule was previously determined to be ±0.5 pH unit over at least a 6-hr period (5). *In vitro* studies have also been conducted to demonstrate a lack of interaction between theophylline and a Heidelberg capsule in simulated gastric and intestinal fluid (5).

Serum Theophylline Assay

Serum theophylline concentrations were determined by a modified high-performance liquid chromatographic method (15). A 0.25-mL serum sample was extracted with 5 mL of a 3% mixture of isopropanol:chloroform after the addition of β-hydroxyethyl-theophylline as internal standard. The organic phase was separated and evaporated to dryness. The dried residue was reconstituted with 0.1 mL of mobile phase and 20 μL was injected into the HPLC. A reverse-phase column (30 cm × 3.9 mm, 10 μm, μBondapak C18, Waters Associates) was employed at ambient temperature. Detection was at 274 nm. The mobile phase consisted of a 95:5 mixture of a 0.023 *M* solution of anhydrous monobasic sodium phosphate containing 0.0025 *M* tetrabutylammonium phosphate (adjusted to pH 4.1) and acetonitrile. The flow rate was 2 mL/min. Standard curves, plotted as drug/internal standard peak height ratio, were prepared over a theophylline plasma concentration range of 0.10–20 μg/mL. Quality control samples were also prepared in human plasma, fortified to contain 0.2, 1.0, and 6.0 μg/mL of theophylline. These samples were stored with the subject samples and assayed along with the subject samples.

Data Analysis

The maximum serum concentration (C_{max}) and time of maximum concentration (T_{max}) were determined by inspection. The area under the serum concentration–time profile to the last sampling point, AUC (35/47 hr), was determined by the linear trapezoidal rule. The fraction of the amount of dose absorbed (F_A) was calculated by the Wagner-Nelson method (16), which employs the equation: $F_A = [C_t + K(AUC_t)]/K[AUC(0-\infty)]$, where C_t and AUC_t are the serum concentration and cumulative AUC at time t, respectively. K is the terminal rate constant estimated from the slope of the ln plasma concentration–time profile for each formulation and each subject. The slope was determined from three to six concentrations, using an unweighted least-squares fitting. The same K was also employed to estimate the area under the curve to time infinity for each data set, computed from $AUC(0-\infty) = (AUC_t) + C_t/K$, where t is the time of the last log-linear concentration.

Statistical Analysis

The statistical analysis was based on a general linear model for replicate design (17). The SAS GLM program was employed to analyze a split-plot design for each group (i.e., achlorhydrics and normals). Dosing sequence was the between factor and subjects within dosing sequence was the main plot. Subplots consisted of week, drug, replication, and all interactions of replication with other main effects. In addition, we calculated estimates of the between and within variances for each drug within each group. Sex differences for the parameters within each group were also evaluated and were found to be insignificant ($P > 0.1$), so sex was not utilized as a variable in the main model. The Student–Newman Keuls test was employed to determine significant differences among the variables. All data for Product *N* were normalized to the 300-mg dose employed for the other dosage forms.

RESULTS AND DISCUSSION

Subject 1 was dropped from the study after the second week for reasons unrelated to the conduct of the study, and her data were not used for the statistical analysis. All other subjects successfully completed all eight dosing phases.

The incorporation of a Heidelberg capsule pH measurement as part of the subject screening process was done to evaluate the correspondence between the capsule reading *in situ* and the pH meter measurement of the gastric fluid aspirate. These measurements were conducted to determine if the capsule could be employed to screen subjects for achlorhydria, and if it would be a reliable indicator of gastric fluid pH during the subsequent 8-week bioavailability study. The capsule pH determinations for the initial 15-min aspirate averaged 1.0 unit lower than the pH meter. A plot of pH meter readings for this first aspirate versus the pH determined *in situ* with the Heidelberg capsules yielded a slope of 0.90 and a Y intercept of 1.3 ($r = 0.98$). The pH's determined by the capsule were quite variable during subsequent aspirations, but this was likely the result of the limited fluid volume present in the stomach as a result of the aspiration. However, the readings obtained from the first aspiration indicate the Heidelberg capsule could be used to identify achlorhydric subjects and to monitor gastric pH during the bioavailability study.

The theophylline standard curves were linear ($r > 0.99$) over a plasma concentration range of 0.1–20.0 μg/mL. Plasma concentrations below 0.1 μg/mL were considered nonreportable. The coefficient of variation (CV%) ranged from 19.8% (0.1 μg/mL) to 3.5% (20.0 μg/mL) for the back-calculated standards. The CV values for the quality control samples were 20.1, 9.6, and 9.8% for the 0.2, 1.0, and 6.0 μg/mL concentrations, respectively.

C_{max}, AUC(35/47 hr), and AUC(0–∞)

Table III summarizes the statistical results for drug product differences within each subject group for the various bioavailability and pharmacokinetic parameters. Figure 1 illustrates the mean serum concentration–time profile for each product in the normal and achlorhydric subjects. The statistical analysis of differences between the achlorhydric and the normal subjects indicated no significant differences ($P > 0.05$) for C_{max} for the four dosage forms and for AUC(0–∞) for Products L and S. However the AUC(0–∞) values were significantly higher in the normal subjects than in the achlorhydric subjects for Product K ($P = 0.026$) and Product N ($P = 0.047$). These data might indicate that a higher dose may be appropriate when initiating therapy in achlorhydric patients compared to patients with normal gastric acidity. However, it must be recognized that differences in the AUC values between the achlorhydric and the normal subject groups could be a reflection of differences in the apparent volume of distribution (V_d) and/or clearance of the-

Table III. Summary of Bioavailability Parameters

Parameter	Subject group	Drug product	Mean[a]	Range	CV%	Achlor/normal (%)
C_{max} (μg/mL)	Normal	L	9.2	6.2–12.0	15.1	
	Achlor	L	8.1	5.5–11.5	22.7	88
	Normal	K	4.9	3.9–5.8	12.9	
	Achlor	K	4.8	3.0–5.9	19.3	98
	Normal	S	2.1	1.5–3.5	22.1	
	Achlor	S	2.1	1.3–3.4	24.5	100
	Normal	N	5.2	3.2–8.7	30.7	
	Achlor	N	4.4	2.2–6.8	23.8	85
		Normal: L > N = K > S				
		Achlorhydric: L > K = N > S				
T_{max} (hr)	Normal	L	0.8	0.5–1.2	38.6	
	Achlor	L	0.9	0.5–2.0	61.9	112
	Normal	K	8.6	4.0–15.0	31.8	
	Achlor	K	8.0	6.0–12.0	19.6	93
	Normal	S	11.1	6.0–20.4	37.2	
	Achlor	S	12.9	6.1–25.1	42.2	116
	Normal	N	5.2	3.2–10.0	37.7	
	Achlor	N	8.0	4.0–15.0	37.0	154
		Normal: S > K > N > L				
		Achlorhydric: S > K = N > L				
Half-life (hr)	Normal	L	8.2	4.1–11.3	26.8	
	Achlor	L	7.3	3.6–10.1	25.2	89
	Normal	K	8.6	4.5–12.7	30.2	
	Achlor	K	7.1	3.9–10.0	23.6	83
	Normal	S	16.2	5.8–28.6	46.4	
	Achlor	S	15.6	8.2–26.3	37.5	96
	Normal	N	8.5	4.4–11.9	25.7	
	Achlor	N	7.8	5.3–10.9	23.3	92
		Normal: S > K = N = L				
		Achlorhydric: S > N = L = K				
AUC (35/47) (μg · hr/mL)	Normal	L	92.1	62.8–129.7	20.7	
	Achlor	L	81.5	53.0–122.1	24.3	88
	Normal	K	93.2	54.7–143.6	22.9	
	Achlor	K	77.8	54.7–100.9	16.4	83
	Normal	S	59.9	35.4–90.3	26.1	
	Achlor	S	62.1	37.1–81.2	22.4	104
	Normal	N	89.7	62.7–125.4	22.0	
	Achlor	N	75.2	53.8–97.1	16.9	87
		Normal: K = L = N > S				
		Achlorhydric: L = K = N > S				
AUC (0–∞) (μg · hr/mL)	Normal	L	97.9	62.7–143.9	23.1	
	Achlor	L	85.3	52.3–134.5	27.0	87
	Normal	K	98.1	55.4–155.6	25.5	
	Achlor	K	79.3	54.0–102.9	17.0	81
	Normal	S	77.5	37.5–137.5	39.2	
	Achlor	S	77.3	48.4–130.8	29.7	100
	Normal	N	93.0	63.1–127.5	23.1	
	Achlor	N	77.3	54.0–100.3	16.9	83
		Normal: K = L = N > S				
		Achlorhydric: L = K = S = N				
Relative bioavailability (Product L used as reference)	Normal	K	1.00	0.82–1.20	11.9	
	Achlor	K	0.95	0.77–1.13	13.3	95
	Normal	S	0.78	0.42–1.28	28.5	
	Achlor	S	0.90	0.77–1.10	12.7	115
	Normal	N	0.96	0.76–1.24	18.3	
	Achlor	N	0.93	0.58–1.17	16.2	97
		Normal: K = N > S				
		Achlorhydric: K = N = S				

[a] Means determined with $N = 14$ and $N = 16$ for achlorhydric and normal subjects, respectively.

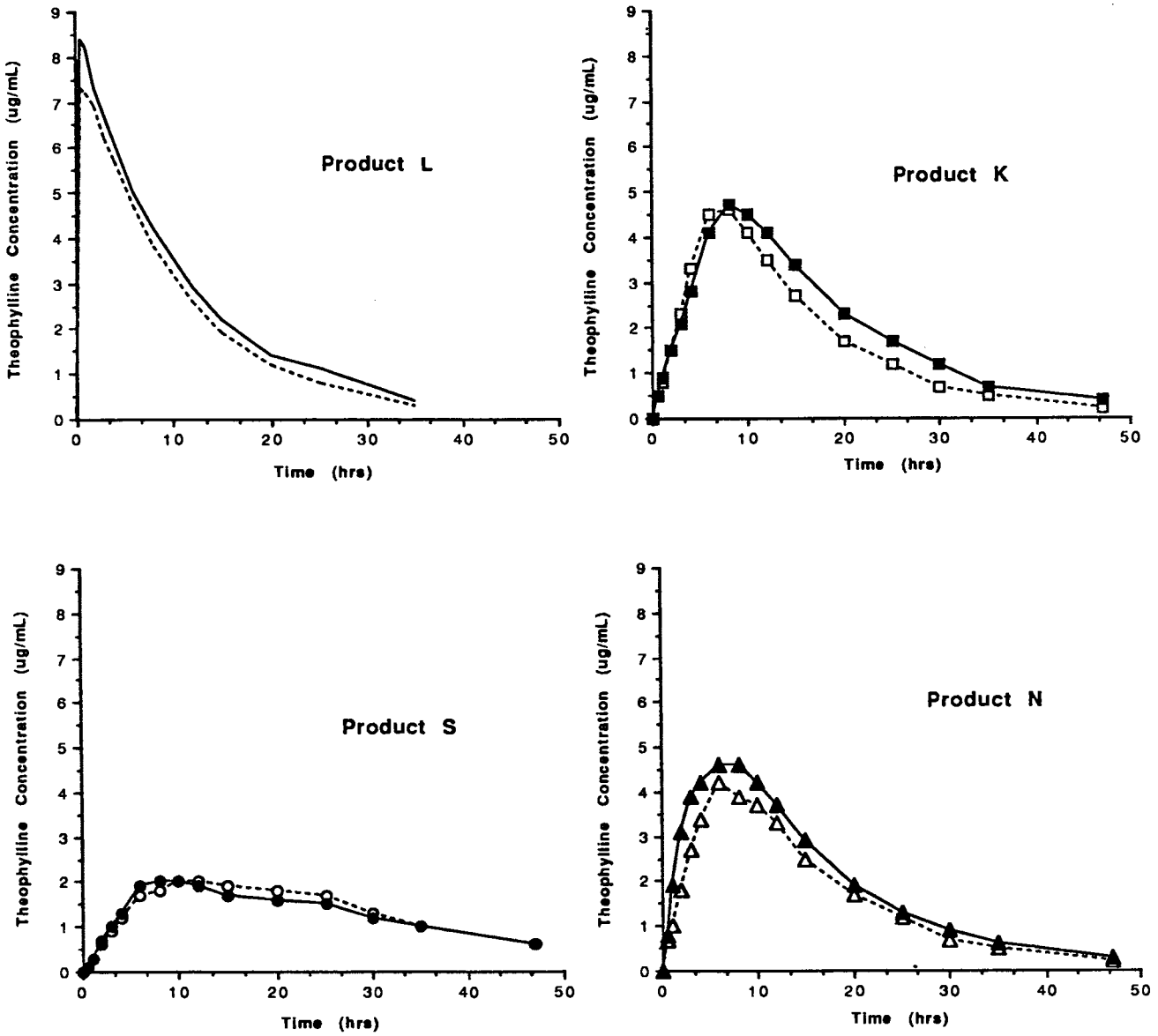

Fig. 1. Mean serum theophylline concentrations following the oral administration of a solution (Product L) and three controlled-release formulations (Products K, S, and N) to eight normal subjects (——; ■, ●, ▲) and seven achlorhydric subjects (---; □, ○, △).

ophylline in the two groups and may not be related to gastric pH. The most important observation to make regarding C_{max} was that the statistical ranking of the four products was the same for both subject groups. One reason for the lower mean AUC(0–∞) values in the achlorhydric group could be the tendency toward shorter terminal half-lives, as summarized in Table III. It should be noted that the apparent longer mean half-life determined for Product S in both groups likely reflects the prolonged absorption from the product which precluded the determination of a true elimination half-life from the available postabsorption data. In addition, the actual extent of absorption for Product S may have been overestimated because of the smaller terminal rate constants employed to extrapolate the AUC to infinity for this product. The AUC to the last sampling time, 35 hr for the liquid and 47 hr for the solid dosage forms, showed Product S to be less completely absorbed than the other three formulations ($P < 0.05$) for both subject groups. In terms of AUC(0–∞), there was no significant difference ($P > 0.05$) observed among the four products in the achlorhydric subjects. However, in the normal group, Product S was significantly lower ($P < 0.05$) than the other three products, while there was no significant difference among Products K, L, and N. In view of the significantly lower value of the AUC(0–∞) for Product S in the normal subjects and the lack of product differences in the achlorhydrics, it would appear that an elevated gastric pH enhances the extent of absorption from Product S. This dosage form has also been shown to exhibit more rapid and greater extent of absorption in humans following administration of a high-fat meal (18).

Since each dosage form was administered twice to each subject, the study design also afforded an opportunity to determine intrasubject variability. Figure 2 summarizes the percentage difference in the replicate AUC(0–∞) values for

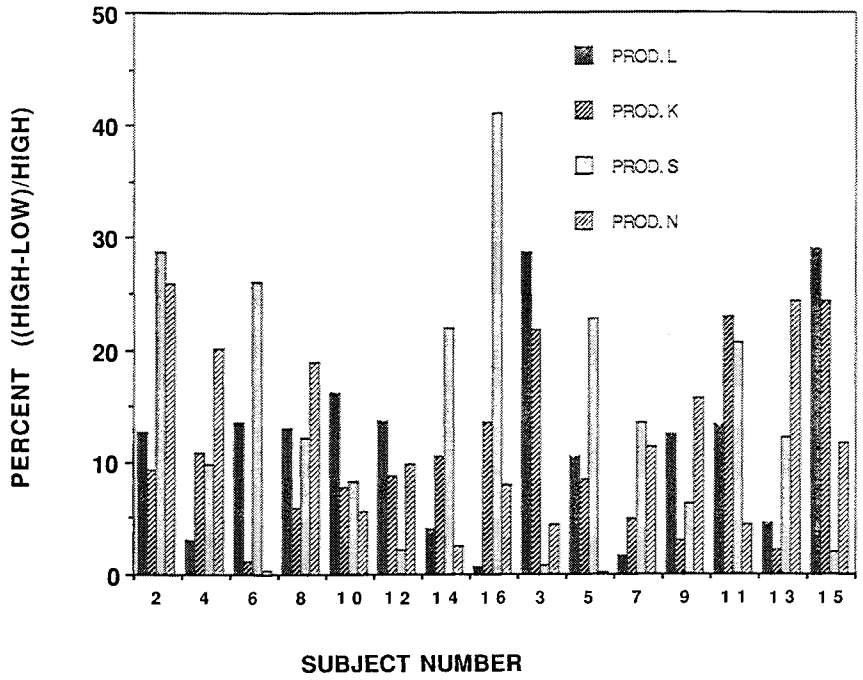

Fig. 2. Intrasubject variability in AUC(0–∞) determined from two separate doses of each product in each subject. Even- and odd-numbered subjects represent subjects with normal and elevated gastric pH, respectively.

each product. In general, most differences were less than 20%. Product S exhibited the greatest intrasubject variability, with differences of 20% or more for 6 of the 15 subjects. The achlorhydric subjects also exhibited a greater intrasubject variability than the normal subjects. Ten of the 14 ratios shown in Fig. 2 which were ≥20% were observed for the achlorhydric subjects.

Relative Bioavailability

The area under the serum theophylline–time profile is a function of the apparent volume of distribution and clearance, as well as the fraction of total dose absorbed. In order to permit a more valid comparison between the normal and the achlorhydric subject groups, the AUC(0–∞) for the three controlled-release products in each subject was divided by the mean AUC(0–∞) for the two doses of the reference liquid (Product L) in each subject. The relative bioavailability of the three controlled-release products ranged from 78 to 100 and 90 to 95% in the normal and achlorhydric subjects, respectively. There was no significant difference ($P > 0.05$) between the achlorhydric and the normal subjects for Product L, K, or N. The higher mean relative bioavailability found for Product S in the achlorhydrics compared to the normals ($P = 0.026$) is indicative of an apparent increase in the extent of absorption for this formulation in the absence of gastric acidity. In addition, these data are consistent with the slightly faster *in vitro* dissolution of Product S during the first hour (14.2 vs 6.5%) and second hour (32.6 vs 22.6%) when simulated intestinal fluid was employed as the dissolution medium instead of simulated gastric fluid for 1 hr followed by simulated intestinal fluid for 11 hr (5). The fact that the differences observed between the normal and the achlorhydric subjects were relatively small could relate to the limited time these products were exposed to the gastric environment. Thus, for theophylline, which is well absorbed throughout the intestine, the extent of absorption from these controlled-release formulations is unlikely to be greatly influenced by gastric acidity if stomach emptying time is not prolonged.

Time of Maximum Serum Concentration and Fraction of the Total Amount Absorbed

A direct comparison between achlorhydric and normal subjects for the time of maximum serum concentration (T_{max}) and fraction of the total amount absorbed (F_A) should be valid, since these parameters should not be affected by intersubject differences in V_d and the relatively small differences in the half-life observed between the two subject groups.

As expected, the solution exhibited the shortest mean T_{max}, which was less than 1 hr for both subject groups. The close correspondence of T_{max} in both groups suggests a similar intrinsic theophylline absorption rate for the achlorhydric and normal subjects. The mean T_{max}'s for each of the four products were significantly different from each other ($P \leq 0.05$) in the normal subjects, while Products K and N exhibited an identical T_{max} in the achlorhydric group. The ranking of the four dosage forms was identical for both subject groups. The most striking difference was with Product N; the mean T_{max} was nearly 3 hr shorter in the normal subjects compared to the achlorhydric group ($P = 0.051$). These data are consistent with the more rapid *in vitro* dissolution of Product N under acidic conditions.

The mean F_A data are illustrated in Fig. 3, and further demonstrate the more rapid absorption of Product N in the normal subjects. When comparing the mean F_A values for Products K and S in the two subject groups, neither product

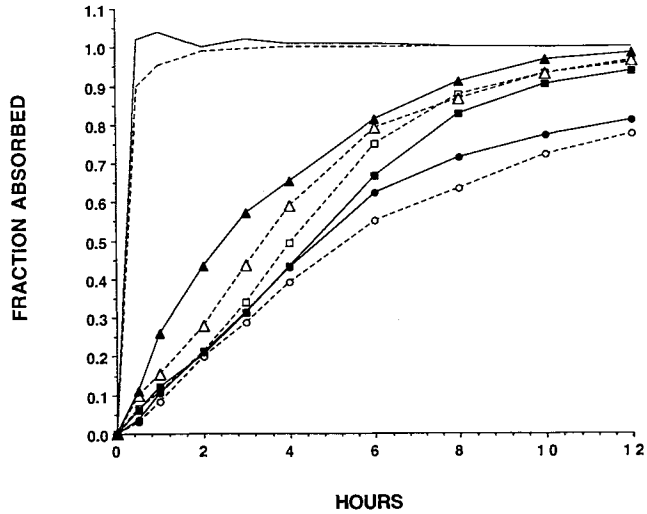

Fig. 3. Mean fraction of the amount absorbed for Product L in achlorhydric (------) and normal (——) subjects: Product K in achlorhydric (□) and normal (■) subjects; Product S in achlorhydric (○) and normal (●) subjects; Product N in achlorhydric (△) and normal (▲) subjects.

differed by more than 13% over the first 10 hr. In contrast, the mean F_A values for Product N were as much as 30–73% higher for the 1- to 3-hr samples in the normal subjects, e.g., F_A = 0.26 versus 0.15 at 1 hr. It should noted that the F_A values for Product S undoubtedly contain some error. The calculation of F_A is based on the use of a terminal rate constant which describes the terminal elimination of the drug. The terminal rate constants for Product S were much smaller than for the other three formulations due to the prolonged absorption of this product. Thus the true values for AUC(0–∞) were probably overestimated, and the F_A plots indicate more rapid absorption than was actually occurring. However, even though the absolute values for the F_A's for Product S may be in error, the relative F_A's comparing the normal and achlorhydric subjects are valid, since both groups exhibited similar apparent prolonged elimination for Product S.

Other Considerations

The mean gastric pH readings for the subjects prior to administration of each of the eight doses ranged from 1.3 to 1.8 for the normal subjects and from 6.3 to 7.3 for the achlorhydric group, with the exception of Subject 12. This subject, who exhibited a normal gastric pH during the screening, had pH's which were 5.5, 6.0, 1.5, <1.0, 5.0, 3.5, 2.0, and 6.0 for weeks 1–8, respectively. Examination of the data from this subject did not reveal any clear relationship between the gastric pH at the time of dosing and the absorption of theophylline from the test products. Since variations from the expected pH were not seen for the other 14 subjects, the variations appeared real and not indicative of a failure of the Heidelberg capsule. The data for Subject 12 were not deleted from the normal subject group since it was noted that the mean T_{max} decreased by only 0.25 hr for Product N, and all other mean values for the normal subject group increased only slightly (≤5%) when the data for Subject 12 were omitted.

Two achlorhydric and two normal subjects in Phase I had undergone an earlier cholecystectomy, and the possibility existed that the absence of bile could effect the dissolution of the drug or alter intestinal transit time. However, recent studies have compared the mouth-to-cecum transit time of a liquid meal in patients with a cholecystectomy and in a control group (19). While the transit times were elevated following cholecystectomy, the differences among the pre- and postcholecystectomy patients and the control group were not statistically significant. It is not possible to determine precisely the effect of a cholecystectomy on the absorption of the dosage forms employed in the present study. However, an examination of the subject ranking with respect to the various bioavailability parameters did not reveal any trend which distinguished these subjects from those with normal bile flow.

It is also possible that gastric fluid volume, as well as gastric pH, may be important in determining the rate of dissolution of the dosage forms *in vivo*. The gastric fluid volumes were determined for each subject at the time of the initial screening procedure involving aspiration of the gastric contents. The mean volume ± SD recovered by continuous gastric aspiration over three consecutive 15-min time periods was 61 ± 51 and 98 ± 38 mL for the achlorhydric and normal subjects, respectively. The difference was significant ($P \leq 0.05$). However, since each dose was administered with 180 mL of water, it is not likely that a volume difference of this magnitude for the gastric secretions would significantly affect the dissolution rates *in vivo*.

In Vivo–in Vitro Relationships

It was of interest to determine if the bioavailability of the three controlled-release products could be predicted in the achlorhydric subjects from the *in vivo/in vitro* correlation in the normal subjects and the *in vitro* dissolution data obtained in simulated intestinal fluid. For each formulation, a pair of plots was constructed relating the fraction of the total amount absorbed (F_A) to the fraction dissolved (F_D). The F_A in normals (F_{A_n}) was compared to the dissolution in the mixed dissolution media ($F_{D_{g+i}}$), while the F_A in achlorhydrics (FA_a) was compared to dissolution of the products in simulated intestinal fluid (F_{D_i}). Figure 4 illustrates the good relationships between the *in vitro* dissolution and the *in vivo* fraction of the amount absorbed data for both subject groups during the first 6 hr. In order for the *in vitro* F_{D_i} to be highly predictive of the *in vivo* F_{A_a}, the pairs of plots for each product would need to be superimposable, and they are not. To test further the *in vitro/in vivo* relationships, predicted values for F_{A_a} were determined. First plots were constructed as shown in Fig. 4 for F_{A_n} versus $F_{D_{g+i}}$ for each product. The resulting slope and intercept values were then used to compute a predicted F_A for the achlorhydric subjects (F_{A_a}) from the experimentally determined *in vitro* dissolution results obtained in the absence of simulated gastric fluid (F_{D_i}). The results are illustrated in Fig. 5. The predicted values for Products K and N underestimated the F_A in the achlorhydric subjects, predicting a slower absorption than was actually observed. In contrast, there was better agreement between

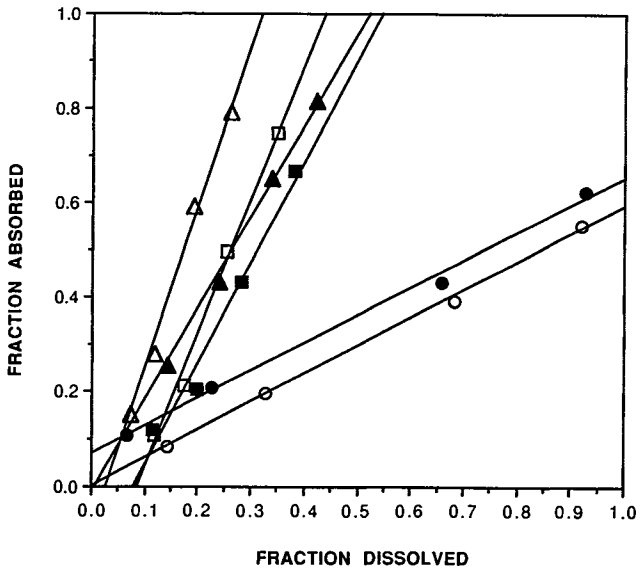

Fig. 4. Mean fraction of the amount absorbed in achlorhydric subjects (F_{A_a}) versus the fraction dissolved *in vitro* in simulated intestinal fluid (F_{D_a}), and mean fraction of the amount absorbed in normal subjects (F_{A_a}) versus the fraction dissolved *in vitro* in simulated gastric fluid, followed by simulated intestinal fluid ($F_{D_{g+i}}$). Product K: (□) F_{A_a}/F_{D_i}; (■) $F_{A_n}/F_{D_{i+g}}$. Product S: (○) F_{A_a}/F_{D_i}; (●) $F_{A_n}/F_{D_{g+i}}$. Product N: (△) F_{A_a}/F_{d_i}; (▲) $F_{A_n}/F_{D_{g+i}}$.

the observed and the predicted data for Product S. These results illustrate the necessity of carefully evaluating any dissolution test data and apparent correlations. Even though Fig. 4 suggested a good *in vitro/in vivo* relationship, the prediction of the effect of pH on the absorption of Products K and N in the achlorhydric subjects was not quantitative. The reasons for the differences shown in Fig. 5 could include: a failure of the *in vitro* system to simulate the gastrointestinal tract adequately with respect to variables such as pH, agitation, transit time, or fluid volume, as well as possible intrinsic differences in theophylline absorption between the achlorhydric and the normal subjects utilized in this study.

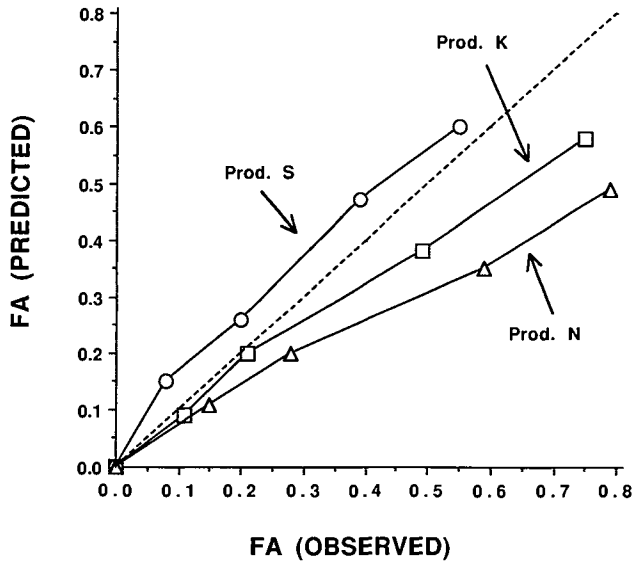

Fig. 5. Predicted fraction of the amount absorbed (F_A) versus observed fraction of the amount absorbed. (----) A perfect correspondence, with a slope of 1.0.

The results of this study have demonstrated qualitative relationships between the *in vivo* bioavailability and the *in vitro* dissolution properties of these controlled-release dosage forms. The rank order of C_{max}, T_{max}, and AUCs for the four formulations was identical in the normal and achlorhydric subjects. This finding suggested that the conduct of bioequivalence testing of these products in subjects with normal gastric pH would not result in the acceptance of a product which would exhibit poor absorption in achlorhydrics subjects. However, among the controlled-release products, the AUC(0–∞) for Product S was significantly lower and the T_{max} for Product N was smaller in the normal subjects, but these parameters were not significantly different in the achlorhydric subjects.

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