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Report

Release and Absorption Characteristics of Novel Theophylline Sustained-Release Formulations: *In Vitro–in Vivo* Correlation

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Five new experimental sustained-release (SR) formulations of theophylline, T-1, T-1-A, T-2, T-2-A, and T-2-E, in a matrix tablet form with a protein were developed. The *in vitro* release of theophylline from these novel experimental formulations and two commercial (Theotrim and Theo-Dur) SR formulations, was studied for 2 hr immersed in simulated gastric fluid TS, followed by an additional 10 hr immersed in simulated intestinal fluid TS. Like Theotrim and Theo-Dur, theophylline release profiles from all the novel experimental formulations were smooth, controlled, and unaffected by changes in the pH and the proteolytic enzyme content of the incubation media. Pharmacokinetic evaluation of T-1, T-1-A, T-2-A, Theotrim, and Theo-Dur was carried out in five dogs and six healthy human volunteers under fasting conditions, using immediate-release aminophylline tablets as controls. Pharmacokinetic analysis by the Wagner–Nelson procedure revealed sustained-release absorption characteristics for all the formulations with the exception of the immediate release aminophylline tablet. For each of the formulations tested, the regression analysis results of the percentage of theophylline absorbed in dogs or humans against the mean percentage released *in vitro*, at the corresponding times, indicated a high correlation. These data imply that the *in vivo* release profiles under fasting conditions in the gastrointestinal tract of dogs and humans may be similar to those in the *in vitro* studies.

KEY WORDS: theophylline; sustained release; release profile; absorption profile; *in vitro–in vivo* correlation.

INTRODUCTION

Sustained release (SR) of drugs in the gastrointestinal (GI) tract following oral administration is the intended rate-limiting factor in the absorption process and, in turn, the bioavailability and therapeutic response (1–3). It is therefore essential in the development stages of oral SR dosage forms to use dissolution methods that allow pharmacokinetic screening of the dosage forms, in particular, the prediction of the absorption rate and the bioavailability. Several approaches to assess *in vitro–in vivo* correlations, particularly for SR dosage forms, have been used. These include plots of the mean percentage released against the mean percentage absorbed (4–8) and statistical moment analysis based on the correlation between the mean residence time (MRT) and the mean dissolution time (MDT) (3,9–12). However, in those reported methods the *in vitro–in vivo* correlations were based on mean data, rather than on individual, and bioavailability was determined only in humans.

This study examined the *in vitro* release and absorption characteristics (in dogs and humans) of theophylline from five novel experimental and two commercial SR formulations and assessed their *in vitro–in vivo* correlation.

MATERIALS AND METHODS

Dosage Forms

Five novel experimental SR formulations of theophylline, T-1, T-2, T-1-A, T-2-A, and T-2-E, were prepared in a matrix tablet form with a protein carrier (13). In its native form, the protein carrier is soluble in electrolyte-free water and has an isoelectric point of 4.63. It can be denatured by various methods, such as heating to 56°C or exposure to alcohols, which produce complete and irreversible denaturation. Theophylline and protein granules at a ratio of 1:0.25 and 1:0.5 were prepared using water as the wetting agent, dried at 120°C for 30 min, sieved, and compressed into T-1-A and T-2-A tablets, respectively, with a manual hydraulic press (Perkin Elmer) under 5000-kg force. Formulations T-1 and T-2 were prepared similarly, except that their granules were dried overnight at room temperature. A mixture of theophylline and protein (1:0.5) was granulated with ethanol (95%), dried overnight at room temperature, and compressed into T-2-E tablets. Binder was not required in the granulation processes since the protein acts in this capacity.

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The tablets each contained 300 mg of theophylline and the respective quantity of protein (Table I).

A commercial immediate-release tablet of 200-mg aminophylline (Sam-On, Israel) and two commercial SR formulations of theophylline (300 mg each), Theotrim (Trima, Israel) and Theo-Dur (Key), were used as references.

In Vitro Dissolution Tests

Dissolution tests were carried out on all the theophylline SR formulations using the rotating basket method (USP Apparatus I) in a 600-ml flask at a constant velocity of 100 ± 5 rpm. Four hundred milliliters of freshly prepared simulated gastric fluid TS was used as the dissolution medium during the first 2 hr, and then replaced by 400 ml of freshly prepared simulated intestinal fluid TS U.S.P. XX (14) for an additional 10 hr, in order to maintain sink conditions throughout the study (15). One-milliliter samples were withdrawn for theophylline determination after 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, and 12 hr and stored overnight at +4°C. Appropriate amounts of the respective dissolution media were immediately added to the vessel to restore the volume lost after each sampling.

Theophylline Analysis

Samples were first centrifuged for 20 min (at 9000 rpm) to remove turbidity due to the enzyme pancreatin and other insoluble particles. A 50-μl aliquot of the supernatant was added to 100 μl of internal standard solution (0.1 mg β-hydroxyethyl theophylline/ml water) and diluted to 1 ml with double-distilled water. Theophylline concentrations in 100-μl samples were determined on an HPLC system, equipped with a C_{18} column (5 μ, 25 × 0.46 cm), at a wavelength of 275 nm, with 14% acetonitrile in double-distilled water at a flow rate of 1.5 ml/min as the mobile phase. The average coefficients of variation for the precision and accuracy were 4.8 and 3.6%, respectively.

Pharmacokinetic Studies

Formulations. Formulations T-1, T-1-A, and T-2-A, Theotrim, and Theo-Dur were evaluated pharmacokinetically in both dogs and healthy human volunteers, using the aminophylline tablet as the reference (16).

Studies in Humans. Six healthy human volunteers, aged 24 to 28 years and weighing 70–95 kg, were selected for the studies. Each subject received one tablet of each formulation (two for aminophylline) in a crossover manner. There was a washout period of 3 weeks between dosings. Tablets were administered at 7:00 AM following an overnight fast. Food was withheld for 5 hr after the administration of each formulation. All caffeinated drinks, beverages, and food were not permitted from 2 days before the beginning until the end of each study. Theophylline plasma concentrations were determined using a modification of an established HPLC method (17).

Studies in Dogs. Three male and two female mongrel dogs, weighing between 19 and 25 kg, were administered in a single 200-mg aminophylline tablet in the first week of study. The sustained-release dosage forms were administered to these dogs in a randomized design, whereby three dogs received one of the experimental formulations and the other two received either Theo-Dur or Theotrim. Drug administration, dosing intervals, and food intake were as described for humans.

Blood Collection. Venous blood samples (4 ml) were taken via an indwelling catheter from the forearm vein (in humans) or the cephalic vein (in dogs) at 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, and 24 hr after each dosing. Additional samples were collected at 2.5, 20, 28, and 32 hr in dogs and at 30, 38, and 48 hr in humans.

In Vivo *Absorption.* The Wagner–Nelson method (18) was used to calculate the percentage of the theophylline dose absorbed:

$$\% \text{ absorbed} = \frac{C_{p,t} + K \cdot AUC_{0\text{-}t}}{K \cdot AUC_{0\text{-}\infty}}$$

where K and $AUC_{0-\infty}$ are the elimination rate constant and the dose-normalized (i.e., to 300 mg of theophylline) area under the theophylline plasma concentration–time profile, respectively, following oral administration of aminophylline tablets.

Table I. *In Vitro* Characteristics of Five Novel Experimental SR Formulations of Theophylline

Formulation	Protein (mg/tablet)	Porosity[a] (n = 6)	Tortuosity (n = 6)
T-1	75	0.80	0.41
T-1-A	75	0.80	0.67
T-2	150	0.69	0.90
T-2-A	150	0.70	1.16
T-2-E	150	0.71	0.62

[a] Mean of two measurements for three tablets.

RESULTS AND DISCUSSION

In GI tract-oriented design and evaluation of drug delivery systems, information concerning the drug, the delivery system, and the specific target site in the GI tract is required (19). In *in vitro* evaluation of oral drug delivery systems, the physiological conditions in the GI tract have to be taken into account, particularly in assessments of pharmacokinetic parameters and *in vitro–in vivo* correlations. The proteolytic enzymes pepsin and pancreatin were added to the dissolution media in order to "simulate" more closely the physiological conditions in the GI tract, such that degradation of the protein carrier could occur. The gastric transit time of a solid dosage form is reported to vary, with a mean of about 2 hr (20–22). Since the aim, with both novel and commercial SR formulations, was to deliver theophylline along the entire GI tract and to simulate more closely the *in vivo* conditions, the mean transit time was used. All of the dissolution tests were therefore carried out for 2 hr in simulated gastric fluid with pepsin, followed by an additional 10 hr in simulated intestinal fluid containing pancreatin.

Mean theophylline release profiles from T-1, T-1-A, T-2, T-2-A, T-2-E, Theotrim, and Theo-Dur are presented in Fig. 1. As Fig. 1 demonstrates, the theophylline release profiles from all of the novel experimental, as well as the commercial SR formulations, were smooth and continuous. They

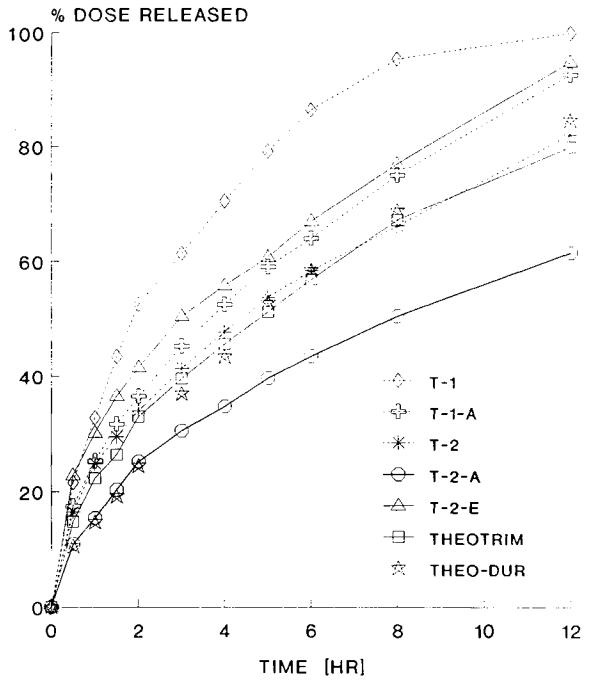

Fig. 1. Mean *in vitro* theophylline release profiles (n = 4) from the novel experimental SR formulations T-1, T-1-A, T-2, T-2-A, and T-2-E, and from two commercial sustained-release formulations, Theotrim and Theo-Dur, into simulated gastric fluid TS (2 hr) and simulated intestinal fluid TS (for a further 10 hr).

were unaffected by changes in pH and proteolytic enzyme content of the medium. These findings may be due to (i) the similar solubility of theophylline (about 12.5 mg/ml) in both of the dissolution media and (ii) the protein matrix cores remained intact and digestion by the proteolytic enzyme was very low, following 12 hr of dissolution. The latter phenomenon does not seem to be consistent with the report on the biodegradability of a hydrophilic protein from a different source in the same dissolution media (23).

Figure 1 also demonstrates the effect of the formulation type on the theophylline release profiles. Varying the ratio between theophylline and protein, the wetting agent in the granulation process, and the method of drying the granules, gave rise to dramatic differences in release profiles. Theophylline was released more slowly from T-2 and T-2-A (ratio 1:0.5) than from T-1 and T-1-A (ratio 1:0.25), respectively. Release from T-2-E (ratio 1:0.5, granulated with ethanol) occurred more rapidly than from T-2 (granulated with water) and T-2-A (heat-dried granules). Theophylline was released more slowly from T-1-A and T-2-A (heat-dried granules) than from T-1 and T-2 (granules dried at room temperature), respectively. Drug release from matrix tablets is known to decrease with decreasing porosity and increasing tortuosity (24). As shown by the results presented in Table I, an increase in the amount of protein in the matrix tablets gave rise to decreased porosity and increased tortuosity. In addition, the wet heat of the granules (T-2-A) or granulation with ethanol (T-2-E) did not affect the porosity of the matrix tablets, as compared to formulation T-2. However, the tortuosity values calculated from the release rates (to be published) indicate that wet heat (T-1-A and T-2-A) resulted in an increase, and granulation with ethanol (T-2-E) gave rise to a decrease in the tortuosity of the matrix tablets, as compared with those of formulations T-1 and T-2. Such results might explain the differences in the release profiles between the various novel experimental SR formulations.

Blood samples for theophylline measurement were collected during the first 12 hr of each *in vivo* study, at the same sampling times as the dissolution tests, in order to give optimal assessment of the *in vitro–in vivo* correlation. The mean absorption profiles of theophylline, calculated by the Wagner–Nelson method, following the administration of three experimental and two commercial SR formulations to dogs and healthy subjects are illustrated in Figs. 2 and 3, respectively. In both dogs and humans, the mean absorption profiles of theophylline following the administration of formulations T-1, T-1-A, and T-2-A were smooth, similar to Theotrim and Theo-Dur, and not affected by the pH gradient in the GI tract. Figures 2 and 3 also relate to the effect of formulation on the absorption profiles and extent of theophylline release from the experimental formulations. In both dogs and humans, absorption occurred more slowly following the administration of formulation T-2-A than following T-1-A, and from T-1-A than from T-1. Pharmacokinetic evaluation of the novel SR formulations has been fully described and discussed elsewhere (16).

Presented in Fig. 4 and Table II are the correlation results obtained from the linear regression analysis between the percentage absorbed in individual dogs and the mean percentage released *in vitro*, at the corresponding times, from the same formulation. Except for formulation T-1, which gave a correlation slope of 1.007 ± 0.051 and a correlation coefficient of 0.907, the correlation slopes of the other formulations ranged between 1.145 ± 0.061 (T-2-A) and 1.334 ± 0.098 (Theotrim), with correlation coefficients

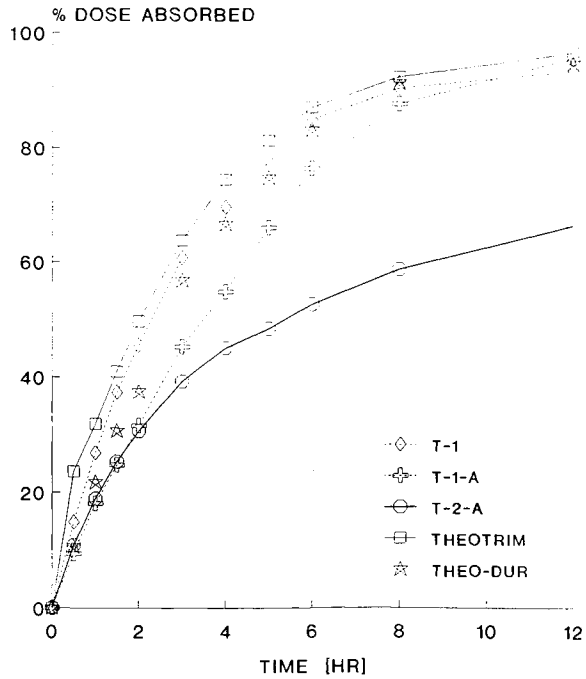

Fig. 2. Mean theophylline absorption profiles following the administration of one tablet (dose of 300 mg) of Theotrim, Theo-Dur, T-1 (n = 4), T-1-A, and T-2-A in a randomized study in five dogs.

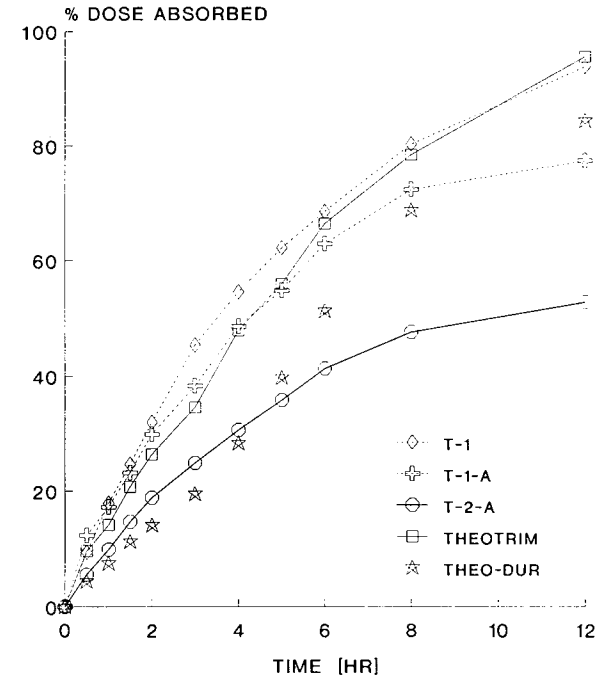

Fig. 3. Mean theophylline absorption profiles following the administration of one tablet (dose of 300 mg) of Theotrim, Theo-Dur, T-1, T-1- A, and T-2-A in a crossover study in six healthy human volunteers.

greater than 0.82. Unlike T-2-A tablets recovered from the dissolution tests in which the perimeter and core of the tablet remained intact, those recovered from feces were split around the entire perimeter, while remaining attached at the core. Possibly, the physiological conditions during fasting conditions in the GI tract of mongrel dogs were slightly more extreme than those in the *in vitro* dissolution tests, leading to the splitting around the entire perimeter, enhanced release, and, in turn, greater absorption.

Table II. The *in Vitro–in Vivo* Correlation Parameters for Three Novel Experimental and Two Commercial SR Formulations Obtained by Linear Regression Analysis

	In vitro–in vivo correlation			
	in vitro in dogs		*in vitro* in humans	
Formulation	Slope (±SD)	r^2 (n)	Slope (±SD)	r^2 (n = 55)
T-1	1.007 (0.051)	0.907 (40)	0.923 (0.037)	0.920
T-1-A	1.260 (0.070)	0.869 (50)	0.940 (0.051)	0.865
T-2-A	1.145 (0.061)	0.870 (54)	0.954 (0.059)	0.831
Theotrim	1.334 (0.098)	0.819 (43)	1.273 (0.057)	0.903
Theo-Dur	1.202 (0.085)	0.825 (44)	0.984 (0.062)	0.826

The results of correlation obtained from the linear regression analysis between the percentage absorbed in individual human subjects and the mean percentage released from the same formulation are presented in Fig. 5 and Table II. As for dogs, the data revealed interindividual variability in absorption for all of the novel experimental and commercial SR formulations of theophylline. A correlation coefficient of 0.903 (n = 55) and correlation slope of 1.273 ± 0.057 were obtained for Theotrim. The mean correlation slope value indicates that under fasting conditions, theophylline release from Theotrim in the GI tract of humans and its consequent absorption were slightly higher than under *in vitro* conditions. For the other formulations, slopes of correlation ranged between 0.923 ± 0.037 (T-1) and 0.984 ± 0.062 (Theo-Dur), with correlation coefficients exceeding 0.83 (n = 55). The high correlations between the percentage

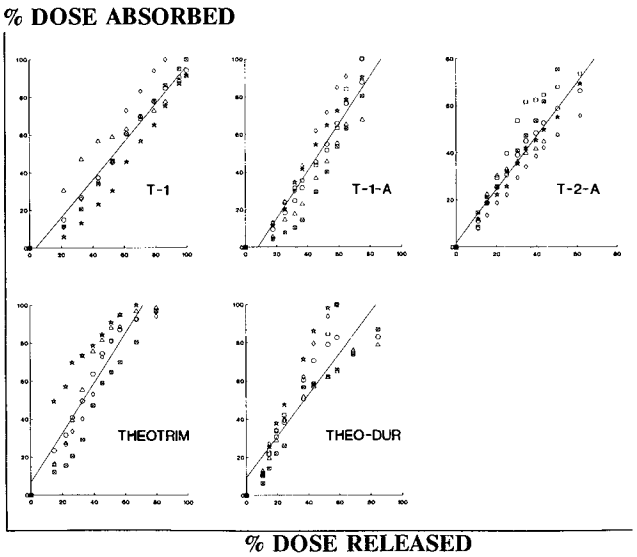

Fig. 4. Plots of the percentage absorbed in individual dogs versus the mean percentage released *in vitro* (n = 4) from Theotrim, Theo-Dur, T-1, T-1-A, and T-2-A. The open circles are the mean data, whereas the other symbols each represent one dog. The lines represent the best correlation, based on linear regression analysis.

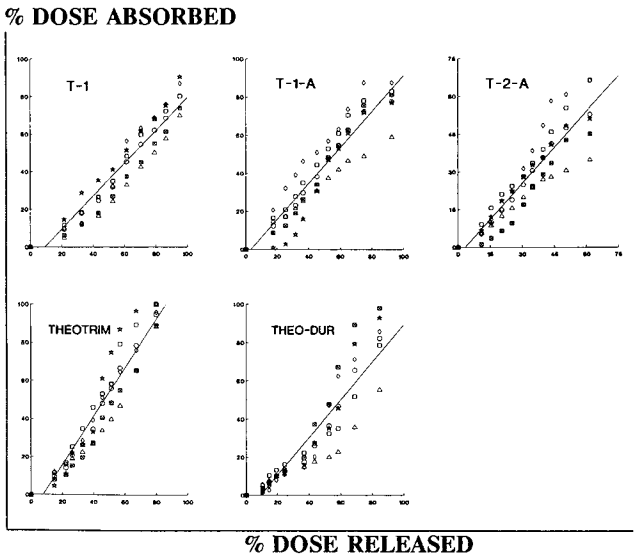

Fig. 5. Plots of the percentage absorbed in individual human volunteers versus the mean percentage released *in vitro* (n = 4) from Theotrim, Theo-Dur, T-1, T-1-A, and T-2-A. The open circles are the mean data, whereas the other symbols each represent one subject. The lines represent the best correlation, based on linear regression analysis.

of theophylline absorbed in individual human subjects and the percentage released *in vitro* from T-1, T-1-A, T-2-A, and Theo-Dur suggest that the theophylline release profiles in the GI tract of humans under fasting conditions and those *in vitro* are similar and that theophylline release is the rate-limiting step in its absorption.

Although theophylline absorption from the GI tract of dogs was higher than the *in vitro* release, correlation between the *in vitro* system and both of the *in vivo* evaluation systems was generally high. The conditions for the dissolution tests seemed to mimic very closely the physiological conditions in the GI tract under fasting conditions, particularly in healthy human subjects.

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