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Bioequivalence of Immediate-release Theophylline Capsules†

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ABSTRACT: A three-way crossover study in 18 healthy male volunteers was conducted to evaluate the bioequivalence of three different 200 mg anhydrous theophylline immediate-release (IR) capsules. The products had not been rated as therapeutically equivalent by the US Food and Drug Administration (FDA) owing to a lack of bioequivalence data. Serum samples were obtained from 0 to 34 h after dosing. Mean time of maximum serum concentration (T_{max}) ranged from 1.3 to 1.4 h. Mean values for the maximum serum concentration (C_{max}) and the area under the serum concentration–time curves (AUC) differed by $< 5\%$ for the three products. The confidence limits for Ln-transformed C_{max} and AUC ranged from ≥ 89 to $\leq 113\%$. It was concluded that the three products were bioequivalent. In addition, the rapid *in vitro* dissolution of these formulations, as well as the reported high solubility and high permeability of theophylline, was predictive of the lack of any bioavailability differences among the three products.

Key words: theophylline; bioequivalence; dissolution; human; Biopharmaceutics Classification System

Introduction

Most generic versions of brand name products are approved for marketing in the US by the Food and Drug Administration (FDA) on the basis of a comparison of the bioavailability of the generic and the brand name product in healthy human subjects. However, in the case of immediate-release (IR) theophylline capsules, marketing occurred prior to the time that human bioequivalence studies were required. Although three formulations of this product were available at the time of this study, none had been compared in a bioequivalence study. Consequently, the FDA did not rate these products as being therapeutically equivalent. Instead, the FDA listed them either as products that lacked data to establish equivalence or as products that had the potential for bioequivalence problems [1]. Recently, there has been an increasing interest in the use of a 'Biopharmaceutics Classification System' to reduce the number of required bioequivalence studies [2]. This approach requires that both drugs and drug products be classified in terms of their solubility, gastrointestinal permeability and rate of dissolution. Drugs that are highly soluble and highly permeable, and that are formulated in rapidly dissolving IR dosage forms, may not require extensive *in vivo* bioavailability testing. Theophylline appears to be such a drug because it has a high aqueous solubility of 8 mg/mL and also has high permeability based on a fraction of the dose absorbed of about 95% [3,4]. In addition, all three products tested in this study dissolved rapidly, i.e. $> 85\%$ dissolved in < 30 min. The present study was conducted to determine whether three different theophylline IR capsules, all manufactured by different firms, were bioequivalent; thus, the use of the 'Biopharmaceutics Classification System' was appropriate for these dosage forms. Since the completion of this study, the manufacturers have discontinued production of the capsules.

Methods

Dosage Forms

Unopened bottles of 200 mg theophylline capsules, which were manufactured by three different firms, were obtained through a local hospital pharmacy. All products were used prior to their expiration date.

In Vitro Dissolution

The three formulations were tested using the USP XXIII dissolution method specified for theophylline capsules. The monograph requires at least 80% dissolution in 60 min using the paddle method at 50

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rpm in water [5]. All three products met this specification.

Study Protocol

A three-way, single dose, bioavailability study was conducted in 18 nonsmoking male volunteers aged between 20 and 33 years, with a mean weight of 77 kg (range 66–93 kg). All subjects were within 10% of their ideal weight for their height. The research followed the tenets of the Declaration of Helsinki promulgated in 1964, and was approved by the University of Tennessee's Institutional Review Board and by the FDAs Risk Involving Human Subject Committee. All subjects provided written informed consent. They were evaluated by a medical history and physical examination and by tests for clinical chemistry (Serum Multichannel Analyzer (SMA) 18/90), complete blood count (CBC), urinalysis and electrocardiogram (ECG).

The three formulations were administered at 1-week intervals in a crossover design. Each subject was randomly assigned to one of six dosing sequences. Each dose was administered along with 180 mL of room temperature (RT) water, after an overnight fast. No food was permitted except for a standard lunch and dinner served 4 and 10 h after dosing, respectively.

Seven millilitre blood samples were obtained prior to dosing and then at 0.33, 0.67, 1, 1.5, 2, 3, 4, 6, 9, 12, 15, 25 and 34 h after dosing. Samples were collected either through an indwelling venous catheter or by direct venipuncture into 7-mL red-topped Vacutainers® (Becton Dickinson, NJ). Blood was allowed to clot at RT for 30 min, centrifuged at $1800 \times g$ and the serum fraction transferred to glass vials and stored at -20°C until analysis.

Chromatographic Analysis

A validated high performance liquid chromatography (HPLC) method, based on Farrish and Wargin [6], utilizing β-hydroxyethyl theophylline as the internal standard, was employed to analyse all samples. Briefly, 0.5 mL of serum was mixed with 0.5 mL of aqueous internal standard (3 μg/mL) and 7.0 mL of 3% isopropanol:chloroform. The mixture was shaken for 20 min at RT. After centrifugation, the organic phase was separated and evaporated to dryness. The residue was reconstituted in 0.1 mL of mobile phase and transferred to sampling vials.

The analyses were carried out using a μBondapak C_{18} column (30 cm × 3.9 mm, 10 μ, Waters Associates, MA) at ambient temperature. The mobile phase was a 95:5 mixture of a 0.023 M solution of pH 4.1 anhydrous monobasic sodium phosphate containing PIC A (Waters Associates, MA) and acetonitrile. Flow rate was 2.0 mL/min and column effluent was monitored at 280 nm. Forty microlitre aliquots of the reconstituted samples were injected. Standard curves were prepared over a range of 0.1–5.1 μg/mL. Control samples contained 0.25, 2.49 and 4.98 μg/mL of theophylline. Quantitation was based on theophylline:internal standard peak height ratios.

Pharmacokinetic and Statistical Analyses

The maximum plasma concentration (C_{max}) and the time to reach the maximum concentration (T_{max}) were determined by inspection of the data. The elimination half-life, the area under the plasma concentration–time curve to 34 h (AUC(0–34)) and the AUC to infinite time ((AUC(0–∞)) were calculated using standard methods [7].

The statistical analysis was carried out using the General Linear Model (GLM) procedure from the SAS statistical package on a VAX 8000 computer. The two, one-sided confidence intervals [8] for C_{max} and AUC(0–∞) were computed, using Ln-transformed data. Current FDA criteria require that these confidence limits be within the range of 80–125%.

Results and Discussion

Chromatographic Analysis

The HPLC assay used in this study was specific for theophylline with no interference from caffeine, theobromine, xanthine, paraxanthine, 3-methylxanthine, uric acid, methyluric acid or 1,3 dimethyluric acid spiked at concentrations of 10 μg/mL. The mean slope for the 20 analytical standard curves in this study was 0.4967 (% coefficient of variation (CV) = 10.1). The precision and accuracy of the assay was determined from the triplicate analysis of the three quality control samples run with each set of subject unknowns. Mean theophylline controls at each of the three levels were within 7% of nominal and had CVs of $\leq 14\%$.

Pharmacokinetic Analysis

All 18 subjects successfully completed the study. Several subjects reported adverse side effects including increased urination, nausea, headache and upset stomach. No significant abnormalities were found in the poststudy clinical evaluations. The mean concentration time profiles for the three products are shown in Figure 1. Mean values of the bioavailability parameters are summarized in Table 1. The maximum difference observed among the three products was only 4% for both AUC(0–∞) and C_{max}, and the differences were not statistically significant ($p > 0.05$) The confidence intervals for the Ln-transformed AUC(0–∞) and C_{max} data ranged from ≥ 89 to $\leq 113\%$ and were well within the range of 80–125%, regardless of which product was utilized as the reference. The values for T_{max}

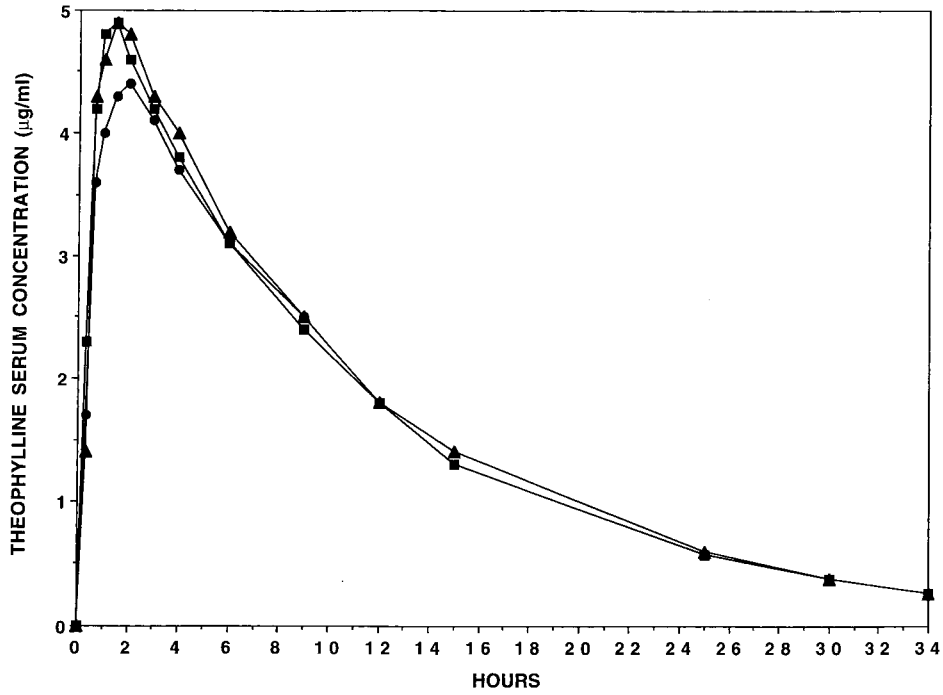

Figure 1. Mean theophylline serum concentration in 18 human subjects who received three different 200 mg theophylline capsules: (■) Product 1; (▲) Product 2; and (●) Product 3

Table 1. Mean (CV%) theophylline pharmacokinetic parameters

Parameter	Product		
	1	2	3
C_{max} (μg/mL)	5.4 (17)	5.5 (19)	5.3 (21)
T_{max} (h)	1.3 (71)	1.4 (55)	1.3 (69)
AUC(0–34) (μg · h/mL)	55.7 (29)	57.1 (30)	54.3 (32)
AUC(0–∞) (μg · h/mL)	59.0 (32)	60.5 (35)	58.1 (37)
Half-life (h)	7.6 (22)	7.5 (24)	7.6 (25)

were essentially identical (range 1.3–1.4 h). The only statistically significant differences ($p < 0.05$) noted among the products were for the mean theophylline serum concentrations at 1, 1.5, 2 and 4 h after dosing. However, none of the concentration differences were $> 17\%$. A significant sequence difference was also observed for AUC(0–∞) and C_{max} ($p < 0.05$). This difference was attributed to one of the three subjects (# 14) in Sequence 5 with a mean half-life for the three treatments of 12.1 h compared with a mean of 7.6 h for all subjects. The mean AUC(0–∞) for Subject 14 was also 93% greater than the mean for all subjects.

Based on the results of this study, it can be concluded that the three theophylline dosage forms are bioequivalent and, thus, may be considered therapeutically equivalent. In addition, the high solubility and high permeability of theophylline, together with the rapid dissolution of these capsules, provides supportive evidence that *in vivo* human bioavailability studies may not be necessary for this drug substance in a rapidly dissolving dosage form.

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