COMPARATIVE BIOAVAILABILITY OF TWO TEST BRANDS OF THEOPHYLLINE; TABLETS AND A REFERENCE QUIBRON®-T/SR UNDER FASTING AND LIMITED FOOD CONDITIONS

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SUMMARY: Comparative bioavailabilities of two test formulations containing 300 mg Theophylline/sustained release tablets were compared with a reference Quibron®-T/SR 300 mg/SR tablet of Mead Johnson. From the concentration-time profiles, the primary pharmacokinetic parameters were evaluated under fasting and under limited food effect conditions in healthy subjects (N=24). The statistical evaluation of the average pharmacokinetic parameters of $AUC_{0\rightarrow\infty}$, $AUC_{0\rightarrow t}$ and C_{max}, demonstrated lack of statistically significant difference between the average pK characteristics from two test formulations versus the reference. The study demonstrated the absence of food effect on Theophylline disposition.

Key Words: Bioavailability, Theophylline, COPD, sustained release.

INTRODUCTION

Theophylline is a bronchodilator used in the treatment of acute and chronic asthma, a chronic obstructive pulmonary disease. The drug is effective within a narrow range of plasma concentration (10-20 μg/ml), while adverse events have been noted when plasma levels exceed 20 μg/ml (1, 2). Theophylline metabolism varies considerably among individual subjects, or dosage forms. Variables such as patient history, diet or consumption of caffeine affect theophylline bioavailability and clearance. Factors that affect clearance also influence bioavailability via their elimination or prolongation of the drug's presence in the body (3). Sustained release product's absorption and bioavailability vary with food (4, 5). Consequently effective and safe therapy requires dose optimization by measuring plasma Theophylline levels, while observing the patient's food habits.

Theophylline has been shown to be extensively metabolized *in vivo*. It is eliminated almost exclusively by the cytochrome P-450 mediated hepatic oxidation, predominantly by 8-hydroxylation to 1,3-dimethyluric acid. The latter pathway accounts for almost half of the total Theophylline clearance (2). In addition, Theophylline is converted to N-demethylated to 1-methylxanthine (1MX) and 3-methylxanthine (3MX). The former is further oxidized by xanthine oxidase to 1-methyluric acid (1MU), which is the only Theophylline 1-demethylation product seen in human plasma and urine.

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This study investigated the ratio of the main pharmacokinetic responses measured from two test drug formulations (300 mg Theophylline/Sustained release tablet) and a reference drug formulation Quibron®-T/SR (300 mg Theophylline/Sustained release tablet) of Mead Johnson. Under both fasting and limited-food conditions, the average pharmacokinetic responses did not exceed the bioequivalence acceptable range.

STUDY PROTOCOL

Each study had an open, randomized, 2x2 cross-over design with two treatment periods. In each of the periods a single oral dose of the corresponding formulation was administered. These periods were separated by a washout period of 11 days.

The sequence in which the subjects received the treatment (test or reference formulation) was determined according to a previously chosen randomized scheme for a balanced 2x2 cross-over design. In each study equal numbers of healthy male non-smoking subjects (n=12/sequence) were randomly assigned to two dosing sequences.

Study 2 under limited-food conditions had an open, randomized cross-over design with three treatments, three periods, and six sequences. In each of three periods a single oral dose of the corresponding formulation was administered. Treatments were separated by washout period of 11 days. On each of the study periods, the subjects will fast, starting 11 hours prior to drug intake. Throughout the study period, smoking, eating food other than that specified in the protocol, and/or intake of alcohol or beverages containing xanthine derivatives were not allowed. The oral dosing of the subjects with the drug products was made with 240 ml water, under the direct supervision of the Clinical Manager, QA Manager and Principal investigators. Immediately after administration each participant's oral cavity was checked with aid of a flashlight and tongue depressant to confirm proper dosing and fluid intake.

The sequence in which the subjects received the treatment (test or reference formulation after a standard breakfast or test after fasting) was determined according to a previously chosen randomized scheme (Figure 1). Equal numbers of subjects (n=4/sequence) were randomly assigned to the six dosing sequences.

BLOOD SAMPLING

Prior to implementation of the studies, the investigators explained the purpose of the studies to the volunteers and that they could withdraw at any time during the study. All the volunteers signed a consent form. Anonymity was secured through use of code numbers.

Figure 1: Design of Study 2.

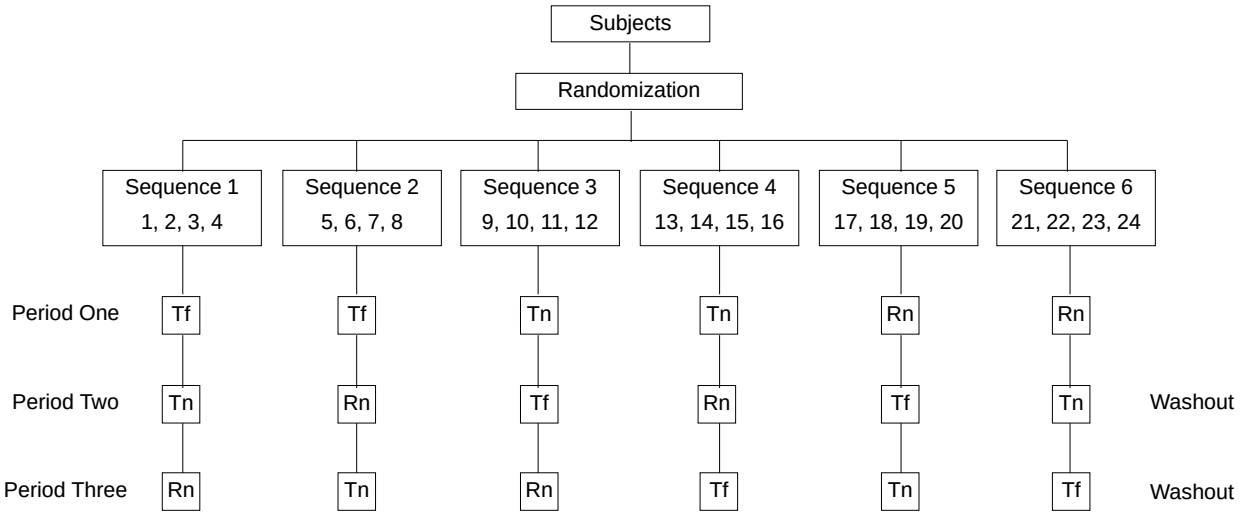


Table 1: Pharmacokinetic parameters for the average of two test brands and the reference Quibron®-T/SR (A) under fasting conditions; (B) under limited food.

A)

Pharmacokinetic Responses	Average of two test formulations (Tablet)		Quibron® -T/SR	
	Average	SD	Average	SD
C_{max} (μg/mL)	4.760	1.398	4.248	1.393
$AUC_{0 \rightarrow t}$ (μg.hr/mL)	70.993	24.114	63.906	28.423
$AUC_{0 \rightarrow \infty}$ (μg.hr/mL)	75.832	24.060	68.959	29.305
T_{max} (hr)	5.560	1.48	5.38	1.31
$T_{1/2}$ (hr)	9.580	2.21	10.29	2.85
$MRT_{0 \rightarrow \infty}$ (hr)	15.760	2.40	16.43	3.31

B)

Pharmacokinetic Responses	Test (Fasting)		Test (Food)		Quibron® (Food)	
	Average	SD	Average	SD	Average	SD
C_{max} (μg/mL)	5.539	0.873	5.655	1.162	5.32	1.163
$AUC_{0 \rightarrow t}$ (μg.hr/mL)	89.749	23.567	92.001	24.492	84.344	20.95
$AUC_{0 \rightarrow \infty}$ (μg.hr/mL)	96.46	24.194	97.235	24.192	90.483	21.056
T_{max} (hr)	5.25	1.03	6.29	1.65	7.04	1.73
$T_{1/2}$ (hr)	9.74	2.40	9.91	2.68	9.36	1.74
$MRT_{0 \rightarrow \infty}$ (hr)	16.71	3.55	17.47	3.5	17.13	2.43

In accordance with the randomization scheme, each volunteer received a prelabeled vial containing the corresponding drug product. About 20 ml aliquot of whole blood was withdrawn from each volunteer prior to drug administration. Blood samples were collected in EDTA blood tubes (2'7.00 ml), and centrifuged for 4 minutes; using polypropylene disposable tips. Plasma was then transferred into screw caped polypropylene tubes, and were immediately stored frozen at -70°C until analysis.

After dosing, about 10.0 ml of whole blood samples were collected at the following time points: 0.25, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 4.00, 5.00, 6.00, 7.00, 8.00, 10.00, 12.00, 14.00, 24.00, 29.00, 33.00, 48.00, 57.00 and 72.00 h after administration of Theophylline (300 mg/tablet). Samples were treated as above. A total of 22 blood samples (about 230 ml of whole blood) were collected in each study period.

DRUG CONCENTRATION MEASUREMENTS

A High-Performance Liquid Chromatographic system coupled with an ultraviolet detector (wave length equals 275 nm) was developed and validated for the determination of Theophylline in human plasma. The method was optimized for purposes of its application to the proposed bioequivalence studies, after a single oral dose of one tablet containing 300 mg/tablet Theophylline.

PHARMACOKINETIC ANALYSIS

The following pharmacokinetic responses for 300 mg Theophylline/tablet were obtained from concentration-time profiles using the appropriate noncompartmental pharmacokinetic model in WIN-NONLIN®.

1. Area under the plasma concentration-time curve from time zero to time t ($AUC_{0 \rightarrow t}$), calculated by the trapezoidal rule, where t is the last time point associated with a measurable plasma level.

Table 2: Statistical evaluation for the main pharmacokinetic parameter indicating the 90% confidence limits.

Pharmacokinetic Responses	Study 1			Study 2		
	Test (Fasting)		Test (Food)		Quibron® (Food)	
	Lower	Upper	Lower	Upper	Lower	Upper
C_{max} (μg/mL)	105.7	118.4	90.9	112.3	95.76	118.3
$AUC_{0 \rightarrow t}$ (μg.hr/mL)	102.4	119.4	91.4	114.4	97.14	121.6
$AUC_{0 \rightarrow \infty}$ (μg.hr/mL)	102.2	117.8	90.92	110.8	97.1	118.3

2. Area under the plasma concentration-time curve from time zero to time infinity ($AUC_{0 \rightarrow \infty}$), where $AUC_{0 \rightarrow \infty} = AUC_{0 \rightarrow t} + C_t/\lambda z$, C_t is the last measurable drug concentration and λz (lambda z) is the elimination rate constant calculated from the terminal segment of the lognormal concentration-time profiles using WINNONLIN®. The terminal or elimination half-life of the drug $t_{1/2}$ and MRT are reported.

3. Peak drug concentration (C_{max}) and the time to peak drug concentration (T_{max}), obtained directly from the data without interpolation.

STATISTICAL ANALYSIS

Prior to analysis it was verified that the distributions of the pharmacokinetic responses were normal. Natural logarithm of the responses with a multiplicative character ($AUC_{0 \rightarrow \infty}$, $AUC_{0 \rightarrow t}$ and C_{max}) was performed. The statistical model analysis of variance (ANOVA), was carried out for the different pharmacokinetic responses ($AUC_{0 \rightarrow \infty}$, $AUC_{0 \rightarrow t}$, C_{max} and T_{max}), which included the following factors: Sequence (Subjects), treatment, period and sequence of administration.

RESULTS

The pharmacokinetic parameters obtained from the analyses of test formulations and Quibron®-T/SR are summarized in Tables 1 and 2, respectively.

Figure 2 shows the dissolution profile of two products while Figures 3 and 4 show the concentration-time profiles after two studies.

Figure 2: The dissolution profile (USP) for the average of two test formulations and for the reference.

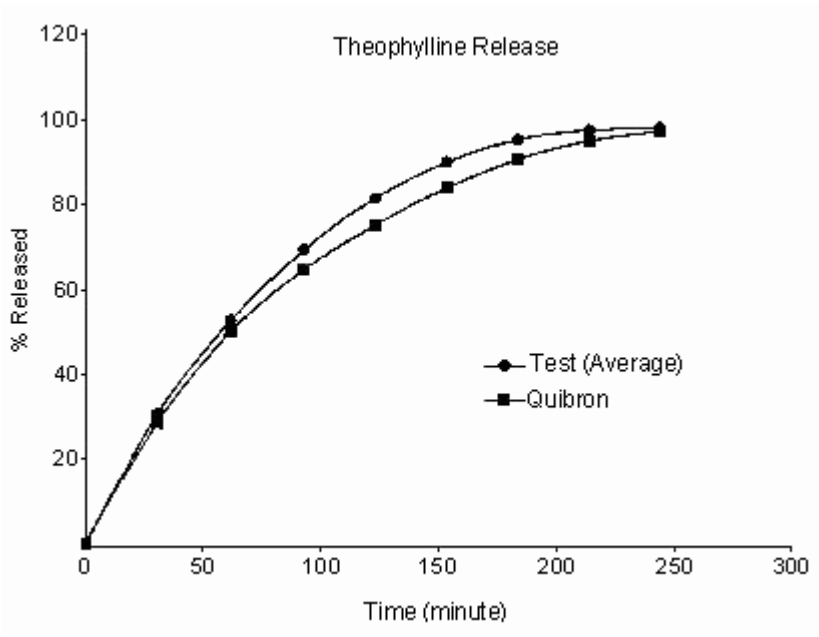
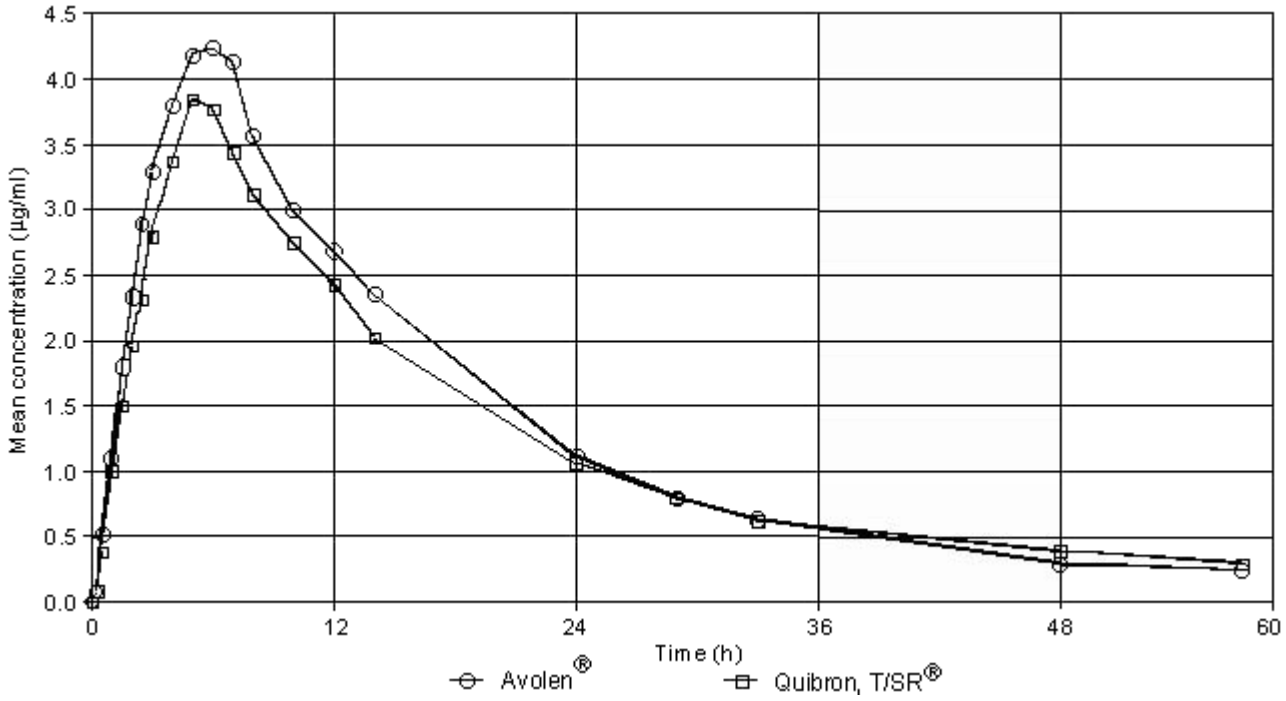


Figure 3: Concentration-time profile (Study 1).

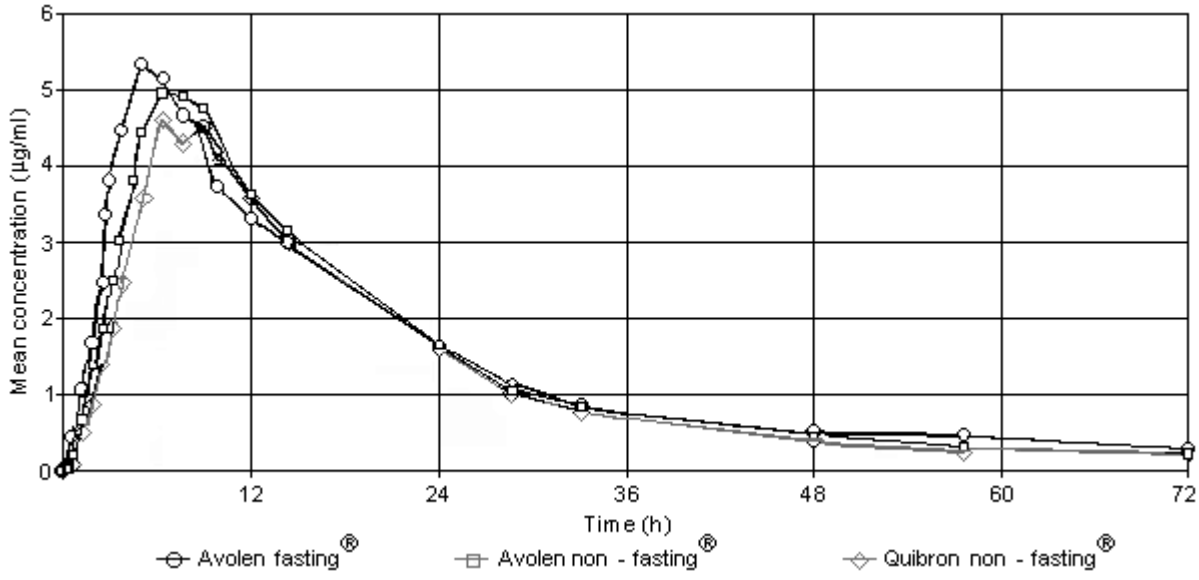


DISCUSSION AND CONCLUSION

Theophylline was well tolerated by the volunteers. The mean and standard deviation of the main pharmacokinetic parameters did not differ significantly, suggesting that the plasma profiles generated by the three products were comparable. The FDA Guidance on evaluation of bioequivalence of drugs after oral administration requires that the 90% confidence intervals of the main pharmacokinetic parameters be within the bioequivalence interval from 80% to 125%.

Study 1 indicated that the two test products are not significantly different under fasting conditions. Study 2 on

Figure 4: Concentration-time profiles (Study 2) obtained from the study under limited food effect.



the other hand indicated that three products are also similar under limited food effect. The analysis of study2 also demonstrated that the test products were not affected by food as indicated by the confidence interval comparing the product under fasting and limited-food states. This supports the findings of Gonzalez and Straughan study that Theophylline is slowly and consistently absorbed from Uni-Dur 24-hour sustained-release form, and food or breaking the tablet does not alter the extent of absorption (6).

The dissolution profiles suggest that the test products release their active ingredients at a relatively faster rate than the reference product. This was also reflected on the *in vivo* results where the test product showed a higher C_{max}, shorter T_{max}, and larger AUC. Accordingly, this study demonstrated that the test products are bioequivalent with the reference product.

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