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COMPARATIVE PHARMACOKINETICS AND BIOEQUIVALENCE OF TWO THEOPHYLLINE 300 MG SUSTAINED RELEASE FORMULATIONS AT ORAL DOSING STEADY-STATE.

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ABSTRACT

The steady-state blood levels of a reformulated Theophylline sustained release tablet formulation (Quibron-T/SR®) were compared with Theo-dur® tablets 300mg SR. Statistical analysis was performed, using the two one-sided tests procedure, on the ln-transformed values of maximum Theophylline plasma concentrations, trough plasma concentrations and Area Under the plasma concentration - time Curve. Data regarding Time to reach maximum plasma concentration were analyzed by analysis of variance on rank transformed values. In every case, the reformulated test treatment was found within the limits required to demonstrate bioequivalence.

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INTRODUCTION

Theophylline formulations are primarily used in the treatment of bronchial asthma, chronic obstructive pulmonary disease and status asthmaticus (1). It can be employed either as a prophylactic drug to relax bronchial smooth muscle in asthmatic patients, or among other drugs in the treatment of prolonged attacks and in the management of status asthmaticus. Theophylline can also be used in the treatment of prolonged apnea sometimes observed in preterm infants.

The recommended oral dosage of Theophylline is achieved by the administration of standard tablet formulations 60 -250mg 3-4 times per day, or by the administration of sustained release (SR) tablets 200 or 300mg twice per day . Literature on Theophylline pharmacokinetics (2,3) shows that the drug has a variable and relatively short plasma half-life. A sustained release dosage form may be able to maintain therapeutic levels of Theophylline, even in subjects who display rapid elimination half-life (e.g. smokers) (4).

The aim of the present study was to compare the steady-state blood levels of two Theophylline SR tablet formulations, a reformulated 300mg SR tablet (Quibron-T/SR®, test formulation) with Theo-dur® tablets 300mg SR (reference formulation). Moreover, this study was designed to verify the findings of the first single dose study done previously on the test formulation (5). The release of Theophylline from the test formulation is controlled by granulation with hydroxypropylcellulose.

MATERIALS AND METHODS

Study design

Ten male, healthy, non-smoking volunteers participated in the study. Their age ranged between 20 to 35 years old and their weight between 65 to 95 Kg. Subjects were asked to abstain from Theophylline containing medications for two weeks and from any other medication for one week before the study started. They all gave oral or written informed consent. Volunteers who displayed hypersensitivity to any of the tested substances, which had history of alcohol abuse, active peptic ulcer, underlying seizure disorders, renal or hepatic deficiency or any other illness that would put into risk their safety or the objective analysis of the drug level, were excluded from the study.

The test formulation, Quibron-T® 300 mg SR, was compared to the marketed Theophylline formulation, Theo-dur®, by means of a steady-state, two-way crossover bioequivalence study, which had a duration of seven days. Volunteers were divided into two groups of five subjects each. The first group (subjects A,B,C,D,E) received the formulations, b.i.d., in the sequence Quibron-T ®300mg SR - Theo-dur® tablet 300mg SR, for seven days. The second group (subjects F,G,H,I,K) received the formulations, b.i.d., in the sequence Theo-dur® tablet 300mg SR - Quibron-T® 300mg SR, for seven days. At twelve hours on day four, the dosage regimen was switched to the second formulation in sequence (reference formulation for the first group and test formulation for the second group).

Volunteers fasted for twelve hours before morning dosing (08:00) on day four and on day seven. They were also asked to abstain from consuming any kind of xanthine containing foods and beverages during the study.

Blood sampling

The collection of samples started (0 hrs) right before morning dosing on day four and seven. In these days, blood samples were also collected at 1, 2, 3, 4, 5, 6, 8, 10 and 12 hours after the morning dose.

Sample analysis

Plasma concentrations of Theophylline were analyzed by a high pressure liquid chromatographic (HPLC) method (Geogarakis; et al., 1990).

Data analysis

Maximum Theophylline plasma concentrations (C_{max}), trough plasma concentrations (C_{min}) and times to reach maximum Theophylline plasma concentrations (T_{max}) were determined directly from individual Theophylline plasma concentration vs. time data. The Area Under the Theophylline concentration - time Curve from 0 to 12 hours (AUC_{0-12}), at steady-state, was calculated using the trapezoidal rule (6). The Fluctuation Index (FI) was obtained using equation 1 (7):

$$FI = (C_{max} - C_{min})/C_{ss} \quad (1)$$

where C_{max} and C_{min} are the peak and trough Theophylline plasma concentrations respectively and C_{ss} the average Theophylline plasma concentration, which was calculated according to equation (2):

$$C_{ss} = AUC_{ss} / \tau$$

where AUC_{ss} is the Area Under the Theophylline plasma concentration vs time curve, over a 12 hour steady state dosage interval and τ is the dosage interval.

The relative bioavailability value (F) was calculated using equation (3):

$$F = \frac{AUC_{0\text{-}12}\ (\text{test formulation})}{AUC_{0\text{-}12}\ (\text{reference formulation})} \qquad (3)$$

where $AUC_{0\text{-}12}$ (test formulation) is the AUC from 0 to 12 h for Quibron-T® SR 300mg and $AUC_{0\text{-}12}$ (reference formulation) is the AUC from 0 to 12 h for Theo-dur ® tablets 300mg SR, at steady state.

RESULTS AND DISCUSSION

The mean ± SD values and CVs of Theophylline plasma concentrations, during the first twelve hours of day four and seven, for the test and reference formulations are given in Table 1. Mean Theophylline plasma concentrations were smaller for Quibron® in all sampling times apart from 5 h. However, both formulations showed large intersubject variation which is reflected by the large CV values. The test formulation displayed CVs ranging from 0.28 to 0.48, compared to 0.38 to 0.52 of the reference formulation. The mean ± SD plasma profiles for the test and reference formulations are given in Figure 1.

The pharmacokinetic parameters for all the subjects and formulations are summarized in Table 2. According to Table 2, the mean C_{max} after multiple dosing of the test formulation is 11.70μg/ml, while the mean C_{max} after multiple dosing of the reference formulation is 12.61μg/ml. Pharmacokinetic parameters were compared using the two one-sided tests procedure, as described by Schuirmann (8). The test was carried out on the natural logarithm transformed values for C_{max}, C_{min} and $AUC_{0\text{-}12}$ (9). The tested interval Hypothesis, H_0, states that the test and reference formulation are *not equivalent.*

TABLE 1: Mean Theophylline plasma concentrations (μg/ml) after multiple oral dosing of Quibron® -T/SR and Theo-dur® tablets 300mg SR.

	Quibron® -T/SR			Theo-dur® 300mgSR		
Time (h)	Mean(μg/ml)	SD	CV	Mean(μg/ml)	SD	CV
0	8.83	4.0	0.47	9.46	4.4	0.46
1	8.45	3.8	0.45	10.39	5.1	0.49
2	8.87	3.2	0.36	10.37	5.2	0.50
3	10.06	3.1	0.31	10.39	5.4	0.52
4	10.69	3.6	0.34	11.11	5.2	0.47
5	10.35	3.5	0.34	9.83	3.7	0.38
6	10.39	2.9	0.28	11.03	5.4	0.49
8	9.90	3.0	0.30	10.82	5.2	0.48
10	8.82	3.7	0.42	9.17	4.6	0.50
12	7.65	3.7	0.48	8.21	4.3	0.52

If null hypothesis is rejected , then the alternative hypothesis, H_1, which states *bioequivalence*, is accepted.

H_0: $X\text{test} - X\text{reference} \leq \ln(0.8)$ or $X\text{test} - X\text{reference} \geq \ln(1.25)$

H_1: $\ln(0.8) < X\text{test} - X\text{reference} < \ln(1.25)$

where $X\text{test} - X\text{reference}$ is the difference between the observed means of a pharmacokinetic parameter. The two sets of one-sided hypotheses were tested by means of two one-sided t-tests, at $\alpha=0.05$ and 8 degrees of freedom ($t_{0.95(8)}=1.8595$). To conclude bioequivalence the following should hold true:

$$t_1 = \{(X\text{test} - X\text{reference}) - \ln(0.80)\}/(s\sqrt{2/n}) \geq t_{0.95(8)} \text{ and}$$
$$t_2 = \{\ln(1.25) - (X\text{test} - X\text{reference})\}/(s\sqrt{2/n}) \geq t_{0.95(8)}$$

where n is the sample size (10) and s is the square root of the "error" mean square

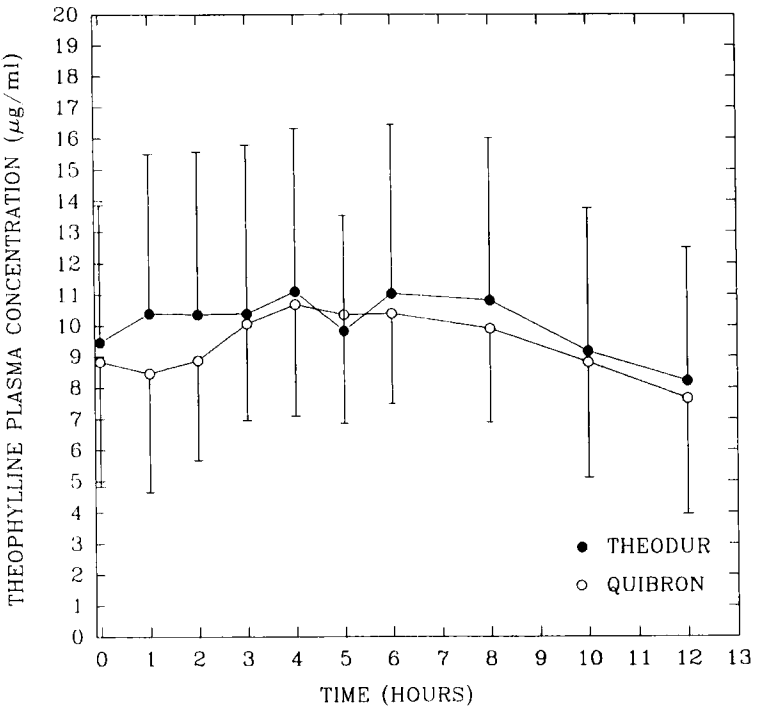

Figure 1: Mean Theophylline plasma concentrations (μg/ml), after multiple oral dosing of Quibron® -T/SR and Theo-dur® tablets 300mg SR. Bars indicate SD.

obtained by the crossover design analysis of variance (ANOVA). The number of the degrees of freedom is that associated with the "error" mean square.

The classic, asymmetric, 90% confidence interval (CI) was also calculated for the difference of the means, $\bar{X}$test -$\bar{X}$reference, following the procedure presented by McGilveray (10).

Values of time to reach maximum Theophylline plasma concentrations (T_{max}), were rank-transformed and then compared, non-parametrically, by ANOVA.

The calculated t_1 and t_2 values for ln(Cmax) of the two one-sided tests procedure were 2.509 and 3.114 respectively which are both greater than $t_{0.95(8)}$=1.8595. Therefore, the alternative hypothesis, stating bioequivalence, is accepted. The 90% CI for the

TABLE 2: Pharmacokinetic parameters, at steady-state, for Quibron® -T/SR and Theo-dur® tablets 300mg SR.

	Subject	Cmax(μg/ml)	Tmax(h)	Cmin(μg/ml)	FI	AUCss(μg.h/ml)
Quibron	A	13.08	5	7.19	0.58	122.5
	B	14.25	10	11.45	0.21	157.3
	C	9.17	6	5.32	0.56	81.9
	D	10.84	6	4.27	0.87	90.6
	E	9.82	4	7.30	0.29	102.9
	F	11.08	5	6.38	0.57	99.4
	G	8.49	2	4.08	0.66	79.1
	H	8.72	3	3.27	0.97	67.3
	I	16.60	4	13.77	0.19	181.2
	K	14.98	4	10.66	0.34	152.7
	MEAN	11.70	4.5*	7.37	0.53	113.5
	SD	2.86	(2,10)	3.51	0.27	38.4
Theo-dur	A	15.84	6	7.03	0.71	148.9
	B	12.36	4	8.67	0.35	126.4
	C	6.86	5	4.96	0.33	69.5
	D	6.80	4	3.05	0.73	62.2
	E	13.70	8	5.43	0.82	121.4
	F	12.54	8	7.50	0.52	117.0
	G	8.54	3	4.63	0.59	78.9
	H	8.39	5	4.93	0.53	77.8
	I	22.20	6	13.89	0.42	236.5
	K	18.90	3	9.59	0.63	176.6
	MEAN	12.61	5*	6.97	0.56	121.5
	SD	5.21	(3,8)	3.15	0.16	54.8

*Median (minimum, maximum)

difference of ln-transformed C_{max} means is (84.2;113.1) (Table 3) and is completely within the 80-125% range of the reference treatment mean value which is required to demonstrate bioequivalence.

The mean trough plasma concentration (C_{min}) for Quibron® is 7.37μg/ml, which is higher compared to that of the test formulation (6.97μg/ml) (Table 2). The difference of the ln-transformed mean values was again tested with two one-sided tests. The values for t_1 and t_2 were 3.770 and 2.624 respectively which are greater than $t_{0.95(8)}$. As a result, the null hypothesis , stating that the formulations are not bioequivalent, was rejected . The 90% CI of the difference of means is (91.4;118.5) which is within the 80-125% range of the Theo-dur® mean value.

The median for the time to reach maximum values (T_{max}) was the same for both the test and reference formulations (5h) (Table 2). The non-parametric examination by means of analysis of variance on rank-transformed values of Tmax indicated that there was no significant difference between treatments at $p>0.30$.

The mean FI were 0.56 for Theo-dur® and 0.53 for Quibron®, The indices are within less than 6% of each other.

The mean (± SD) values for the Area Under the Curve, estimated at steady-state, are 113.49 (38.4) for the test formulation and 121.52 (54.8). It should be noted that although the reference formulation displayed greater mean AUC, it also demonstrated greater intersubject variability . The CV for the reference formulation was 0.45, compared to that of 0.30 of the test formulation. The t_1 and t_2 calculated values for the two one-sided tests are 2.989 and 3.984 respectively, and are both greater than $t_{0.95(8)}$, which is required in order to demonstrate bioequivalence. The 90% CI for the difference of means is (86.0;109.1) and is completely within the 80-125% interval of the reference treatment mean. Based on equation (3) it is possible to calculate the relative bioailability F. The mean relative biovailability , estimated at steady-state, was 0.99 with an SD of 0.23. The lower F value was 0.82 and the larger was 1.24.

Overall, the performance of the reformulated test treatment, as depicted by the two one-sided tests procedure, was found within the limits required to demonstrate bioequivalence. Specifically, the t_1 and t_2 values calculated for the difference between

TABLE 3: Statistical analysis data from the two one-sided tests procedure.

Parameter	Ratio of Means (%)	t1	t2	90% Confidence Intervals
$\ln(AUC_{0-12})$	96.9	2.989	3.984	86.0 - 109
$\ln(C_{max})$	97.6	2.509	3.114	84.2 - 113
$\ln(C_{min})$	104.0	3.770	2.624	91.4 - 118
rank(T_{max})		$p > 0.30$		

mean C_{max}, mean C_{min} and mean AUC_{0-12}, were, in all cases larger than the required t value. Moreover, the 90% Confidence Intervals were completely within the 80-125% bounds for C_{max}, C_{min}, AUC_{0-12}. The non-parametric analysis for T_{max} demonstrated not significant differences at $p<0.05$. Mean values of Fluctuation Indices are within 6% of each treatment. The mean relative bioavailability was found to be 0.99. In addition the test formulation displayed lower intersubject variation, as shown by the smaller CV values. In conclusion, bioequivalence of reformulated Quibron®-T/SR and marketed Theo-dur® tablet 300mg SR has been demonstrated.

REFERENCES

1. T.W. Rall, Central Nervous System Stimulants (Continued). in Gilman, A.G., Goodman,L.S., Rall, T.W., Murad, F., The Pharmacological Basis of Therapeutics,Macmillan Publishing Company, New York, 1985, pp 598-601.

2. R.I. Ogilvie, Clin. Pharmacokin., 3,267-293 (1978).

3. L. Hendeles, M. Weinberger, and G. Johnson, Clin. Pharmacokin., 3,294-312 (1978a).

4. S. Riegelman, and J.W. Jenne, Chest, 78,250-251 (1980).

5. M. Georgarakis, A. Panagopoulou, P. Hatzipantou, T. Iliopoulos, M. Kondylis, D. Grekas, Drug Dev. Ind. Pharm. 16,315-329 (1990).

6. R.J. Tallarida, R.B. Murray, "Manual of pharmacologic calculations". Second Edition, Springer-Verlag, New York, 1987, pp. 77-81.

7. Z. Hussein, M. Bialer, M. Friedman and I. Raz, Biopharm. Drug Disp. 8,427-435 (1987).

8. D.J. Schuirmann, J. Pharmacokin. Biopharm., 15,657-680 (1987).

9. K.K. Midha, E.D. Ormsby, J.W. Hubbart, G. McKay, E.M. Hawes, L. Gavalas, and I.J. McGilveray, J. Pharm. Sci., 82,138-144 (1993).

10. I. McGilveray, Bioequvalence: A Canadian Regulatory Perspective, in Welling, P.G., Tse F.L., Dighe, S.V. (Eds), "Pharmaceutical Bioequivalence", Marcel Dekker,Inc., NewYork, 1991, pp. 381-418.