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In Vivo Performance of a Multi-particulate Matrix, Controlled-Release Theophylline Preparation

Kok-Khiang Peh and Kah-Hay Yuen

School of Pharmaceutical Sciences, University of Science Malaysia, 11800 Penang, Malaysia

ABSTRACT

An experimental multiparticulate matrix sustained-release theophylline preparation was evaluated in vivo, in comparison with Neulin-SR®. Twelve healthy volunteers participated in the study, conducted according to a randomized, two-way cross-over study design. The preparations were compared using the pharmacokinetic parameters, peak serum concentration (C_{max}), total area under the serum concentration-time curve ($AUC_{0-\infty}$), time to reach peak serum concentration (T_{max}), and time for 50% dose absorption in vivo ($T_{50\%}$). A statistically significant difference was observed in the T_{max} and $T_{50\%}$ values ($p < 0.05$) but not in the logarithmic transformed values of C_{max} and $AUC_{0-\infty}$. These findings indicate that the two preparation are comparable in the extent but differ in the rate of absorption, with the experimental preparation being more sustained. In addition, the elimination rate constant (K_e), elimination half-life ($t_{1/2}$), and apparent volume of distribution (V_d) of the drug were calculated. There was no statistically significant difference between the K_e, $t_{1/2}$, and V_d values obtained from the data of the two preparations. Moreover, the values obtained are comparable to those reported in the literature. A satisfactory correlation was also obtained between the mean in vivo absorption and mean in vitro dissolution data ($p < 0.001$) for both preparations.

INTRODUCTION

In vitro dissolution testing offers a cheap and convenient method of evaluation during development of sustained-release preparations. Various important formulation variables affecting the drug dissolution can be investigated, leading to the formulation of a satisfactory product with the desired in vitro drug release characteristics. However, satisfactory in vitro release may not be a real index of the product's in vivo performance. To be acceptable for therapeutic use, the product has to be evaluated on humans, usually using healthy volunteers (1). It is also essential to compare the product with a reference preparation which has been proven to be safe and effective.

In a previous report (2), we have described the development of a multiparticulate matrix, controlled-release theophylline formulation based on in vitro dissolution studies. A preparation consisting of 50% theophylline, 20% microcrystalline cellulose, and 30% glyceryl monostearate was found satisfactory. Drug release was sufficiently sustained and essentially independent of pH and agitation rate. Moreover, the drug release was found to be stable after storage for 6 months. In view of the satisfactory in vitro release characteristics, the present study was therefore conducted to evaluate the in vivo performance of the experimental preparation using healthy human volunteers. In this regard, an established proprietary preparation, Neulin-SR® was used for comparison. The in vivo absorption profiles of the two preparations calculated using the Wagner–Nelson method (3) were further compared. In addition, association between the in vitro and in vivo data was determined for both preparations.

MATERIALS

Neulin-SR 250 mg tablets—batch no.: 9378B, manufacturing date:15/1/94, expiry date:15/1/96, registration no.: PBKD/872256, product of 3M Australia—were purchased commercially. Theophylline anhydrous and β-hydroxyethyltheophylline anhydrous standards were obtained from Sigma Chemical Co., USA. Tetrahydrofuran, HPLC grade was obtained from Lab-Scan Analytical Sciences Ltd., Ireland. Both HPLC grade, chloroform and glacial acetic acid 100% were obtained from E. Merck, Darmstadt. Isopropyl alcohol, A.R., was obtained from Ajax Chemical Ltd., England. Sodium acetate anhydrous, A.R., was purchased from R&M Marketing, England.

METHODS

In Vivo Study Protocol

Twelve healthy, nonsmoking adult male volunteers between 29 and 43 years old (mean = 36 years, *SD* = 6 years), with heights from 149 to 189 cm (mean = 170 cm, *SD* = 11 cm) and weighing from 52 to 82 kg (mean = 68 kg, *SD* = 11 kg), participated in the study. Written informed consent was obtained from the volunteers after explaining the nature and purpose of the study. All were judged to be healthy and were not receiving any medication during the study period. The study protocol was approved by an Ethics Committee on Bioavailability Studies. The volunteers were randomly divided into two groups of six each and administered the preparations according to the following schedule:

	Period	
Group	I	II
1	Neulin-SR	Experimental preparation
2	Experimental preparation	Neulin-SR

On the first trial period, each volunteer in group 1 was given 1 tablet of Neulin-SR (250 mg); those of group 2, 1 capsule of the experimental preparation equivalent to 250 mg of the drug. After a washout period of 1 week, the volunteers received the alternate preparation. Both preparations were administered with 150 ml of water in the morning at 9:00 a.m. after a 12-hr overnight fast. Food and drinks were withheld for at least 2 hr after dosing. Lunch and dinner, comprising chicken with rice, were served at 4 and 9 hr after drug administration and water was given ad libitum. The volunteers were requested to abstain from alcohol and xanthine-containing food or beverages 24 hr before and during the entire study period. Venous blood samples of 5-ml volume were drawn via an indwelling cannula from the forearm into plain vacutainers at 0 (before dosing), 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 14, 18, and 24 hr after dosing. The 36-hr blood sample was taken by direct venipuncture. The blood samples were allowed to stand for 2 hr. After clot retraction, the samples were centrifuged at 3500 rpm for 15 min and the serum was transferred to separate glass containers and kept frozen until analysis.

Serum Theophylline Concentration Analysis

The serum samples were analyzed using a reversed-phase high-performance liquid chromatographic (HPLC)

method described by Yuen et al. (4). The HPLC system comprised a Jasco PU-980 Intelligent HPLC Pump, a Jasco UV-975 Intelligent UV/VIS Detector, a Rheodyne six-port injection valve equipped with a 20-μl sample loop, and a Hitachi D-2500 Chromato-Integrator. The column used was a Whatman Partisil 5-ODS 3 (5 μm, 100 × 4.6 mm ID) fitted with a Upchurch refillable guard column (Perisorb® RP-18, 30–40 μm pellicular stationary phase).

The standard curve was prepared by spiking drug-free serum with a known amount of theophylline in a concentration range of 1–10 μg/ml. Recovery, accuracy, within-day, and between-day precision studies ($n = 6$) were performed using these serum standards. In addition, detector response was found to be linear over a concentration range of 1–64 μg/ml. The recovery values for theophylline was 92.7% at 2.0 μg/ml and 90.5% at 10.0 μg/ml, while that of β-hydroxyethyltheophylline was 91.6%. The coefficients of variation for these recovery values were 5.7%, 2.4%, and 2.7%, respectively. The within-day percent error values were 3.8% at 1.0 μg/ml, 4.1% at 4.0 μg/ml, and 2.7% at 8.0 μg/ml, while the between-day percent error values were 7.8%, 0.1%, and 1.4% at these three concentrations. For the precision studies, the values of within-day coefficients of variation were 3.0% at 1.0 μg/ml, 2.5% at 4.0 μg/ml, and 1.5% at 8.0 μg/ml, while those of the between-day were 3.9%, 1.4%, and 3.1% at these concentrations. The sensitivity of the assay method was approximately 0.1 μg/ml.

Pharmacokinetic Parameters Analysis

The pharmacokinetic parameters—namely peak serum drug concentration, C_{max}, time to reach peak serum drug concentration, T_{max}, and total area under the serum drug concentration–time curve, $AUC_{0-\infty}$—were estimated from the serum concentration–time profiles of the two preparations for each volunteer. The values of C_{max} and T_{max} were obtained directly from the measured serum concentration data (5). The $AUC_{0-\infty}$ was calculated by adding the area from time zero to the last sampling time t, AUC_{0-t}, and the area from time t to infinity, $AUC_{t-\infty}$. The former was calculated using the trapezoidal rule; and the latter by dividing the last measurable serum drug concentration with the elimination rate constant (K_e). In all cases, the $AUC_{t-\infty}$ was found to be less than 20% of the $AUC_{0-\infty}$. The K_e was estimated from the terminal slope of the individual serum concentration–time curves after logarithmic transformation of the serum concentration values and application of linear regression (6). On the other hand, the elimination half-life, $t_{1/2}$, was calculated from the quotient $\ln 2/K_e$, while the apparent volume of distribution, V_d, was calculated as $Dose/(AUC \cdot K_e)$.

The Wagner–Nelson method (3) was employed to calculate the in vivo absorption profiles of the two preparations for each individual. The time for 50% of the dose to be absorbed, $T_{50\%}$, was then estimated from the individual in vivo absorption versus time profiles.

In addition, correlation between the in vitro dissolution and in vivo absorption times for 10%, 20%, 30%, 40%, 50%, 60%, 70%, and 80% of drug released/absorbed was determined for the two preparations.

Statistical Analysis

All the results are expressed as mean ± standard deviation, *SD*. For each of the parameters, $AUC_{0-\infty}$, C_{max}, K_e, $t_{1/2}$, V_d, and $T_{50\%}$, the values obtained for the two preparations were analyzed statistically using an analysis of variance procedure (ANOVA) which distinguishes effects due to group, subjects/group, period, and treatment (7). The $AUC_{0-\infty}$ and C_{max} values were logarithmic transformed prior to the analysis. On the other hand, the T_{max} values of the two preparations were compared using the Wilcoxon signed-rank test for paired samples. A statistically significant difference was considered when $p < 0.05$.

RESULTS AND DISCUSSION

The mean serum theophylline concentration versus time profiles of the two preparations are shown in Fig. 1. Both profiles are reflective of a slow and sustained rate of drug absorption, and serum drug concentration was still detectable at 36 hr. However, the serum levels of the experimental preparation appeared to be more uniform in comparison. The numerical values of C_{max}, T_{max}, and $AUC_{0-\infty}$ of the individual subjects obtained with Neulin-SR and the experimental preparation are presented in Table 1. The mean T_{max} values for Neulin-SR and the experimental preparation were 5.3 hr (*SD*, 2.4 hr) and 8.8 hr (*SD*, 2.7 hr), respectively, indicating a relatively slower absorption rate for the experimental preparation. A statistically significant difference was observed between the T_{max} values ($p = 0.0034$, $p < 0.01$) of the two preparations. The corresponding mean C_{max} values for Neulin-SR and the experimental preparation were 4.78 μg/ml (*SD*, 1.09 μg/ml) and 3.92 μg/ml (*SD*, 0.65 μg/ml), respectively, with the mean

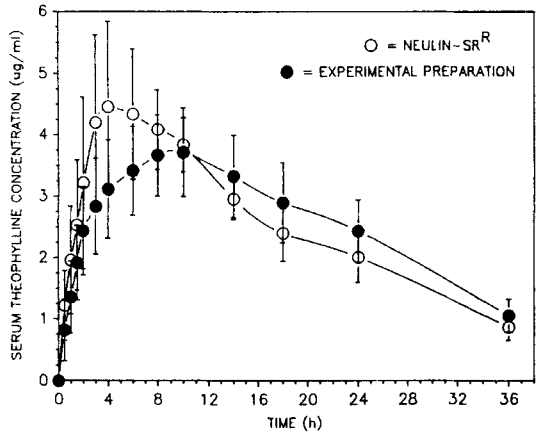

Figure 1. Mean serum theophylline concentration versus time profiles of Neulin-SR and the experimental preparation. Mean ± *SD*; $N = 12$.

C_{max} value of the latter being comparatively lower. However, no statistically significant difference was observed between the C_{max} values ($p = 0.0521$, $p < 0.05$) of the two preparations. In the case of the parameter $AUC_{0-\infty}$, the values obtained with the two preparations were closely similar. Neulin-SR has a mean value of 104.60 hr · μg/ml (*SD*, 13.69 hr · μg/ml), while the experimental preparation has a mean value of 108.82 hr·μg/ml (*SD*, 18.08 hr·μg/ml). No statistically significant difference was observed between the $AUC_{0-\infty}$ values ($p = 0.4307$, $p > 0.05$).

The 90% confidence interval for the ratio of the logarithmic transformed $AUC_{0-\infty}$ values of the experimental preparation over those of the Neulin-SR was also calculated and found to lie between 0.96 and 1.12. This is within the acceptable equivalence interval of 0.80–1.25 (8–10). On the basis of the results obtained from the above analysis, it can be concluded that the two preparations are comparable in the extent but differ in the rate of absorption, with the experimental preparation achieving a more sustained rate of absorption.

The numerical values of the pharmacokinetic parameters K_e, $t_{1/2}$, and V_d of the two preparations are given in Table 2. The values of all three parameters for both preparations were closely similar and were not significantly different statistically ($p > 0.05$). Moreover, the mean values obtained were in good agreement with those reported in the literature for healthy, nonsmoking adults (11,12).

Though a lag time in absorption has been observed by Benedikt et al. (13) and Yuen et al. (4) with their sustained-release theophylline preparations, no lag time in absorption was observed in our preparation. This could be attributed to the difference in the mechanism of drug release between our preparation and those of the above workers. In our preparation, the drug was dispersed in a nonsoluble matrix to control the rate of drug

Table 1

Individual Numerical Values of C_{max}, T_{max}, and $AUC_{0-\infty}$

	Neulin-SR			Experimental Preparation		
Subject	C_{max} (μg/ml)	T_{max} (hr)	$AUC_{0-\infty}$ (μg · hr/ml)	C_{max} (μg/ml)	T_{max} (hr)	$AUC_{0-\infty}$ (μg · hr/ml)
1	5.02	6.0	95.28	3.83	10.0	98.33
2	4.20	4.0	92.46	2.67	10.0	82.87
3	3.10	10.0	102.85	3.91	14.0	125.90
4	4.67	6.0	120.44	4.32	10.0	122.82
5	5.10	6.0	103.02	4.26	10.0	121.66
6	6.33	4.0	117.81	3.49	6.0	105.58
7	4.78	3.0	96.48	4.64	8.0	130.30
8	4.73	4.0	109.44	3.80	3.0	95.00
9	4.99	4.0	116.98	4.76	8.0	130.31
10	7.00	3.0	126.93	3.76	8.0	117.94
11	3.86	10.0	83.77	2.99	8.0	82.51
12	3.55	4.0	89.71	4.66	10.0	92.65
Mean	4.78	5.3	104.60	3.92	8.8	108.82
SD	1.09	2.4	13.69	0.65	2.7	18.08
CV%[a]	22.80	45.3	13.09	16.67	30.7	16.62

[a]Coefficient of variation.

Table 2

Individual Numerical Values of K_e, $t_{1/2}$, and V_d

	Neulin-SR			Experimental Preparation		
Subject	K_e (hr^{-1})	$t_{1/2}$ (hr)	V_d (liter/kg)	K_e (hr^{-1})	$t_{1/2}$ (hr)	V_d (liter/kg)
1	0.0733	9.5	0.6754	0.0697	9.9	0.6882
2	0.0720	9.6	0.4941	0.0650	10.7	0.6107
3	0.0710	9.8	0.4175	0.0672	10.3	0.3604
4	0.0624	11.1	0.4057	0.0716	9.7	0.3467
5	0.0794	8.7	0.4562	0.0764	9.1	0.4014
6	0.0823	8.4	0.4029	0.0676	10.3	0.5473
7	0.0600	11.6	0.7712	0.0640	10.8	0.5353
8	0.0650	10.7	0.5168	0.0640	10.8	0.6047
9	0.0601	11.5	0.6838	0.0583	11.9	0.6328
10	0.0611	11.3	0.4605	0.0611	11.3	0.4956
11	0.0830	8.3	0.4385	0.0590	11.7	0.6263
12	0.0736	9.4	0.6107	0.1100	6.3	0.3956
Mean	0.0703	10.0	0.5278	0.0695	10.2	0.5204
SD	0.0085	1.2	0.1257	0.0138	1.5	0.1184
CV%[a]	12.09	12.0	23.82	19.86	14.7	22.75

[a]Coefficient of variation.

release. Hence, the drug exposed on the pellet surface was readily available for dissolution and absorption. In comparison, the pellets used in the studies of Benedikt et al. (13) and Yuen et al. (4) were coated with a rate-controlling membrane. As such, a lag time was needed for the gastric fluid to penetrate the coat and dissolve the drug, followed by diffusion of the dissolved drug out of the coat for absorption.

The mean in vivo theophylline absorption versus time profiles of the two preparations calculated using the Wagner–Nelson method (3) are presented in Fig. 2, and their mean in vitro dissolution profiles are shown in Fig. 3. In accord with the serum profiles shown in Fig. 1, the plots in Fig. 2 showed that the experimental preparation has a more sustained rate of absorption compared to Neulin-SR. The mean $T_{50\%}$ values estimated for

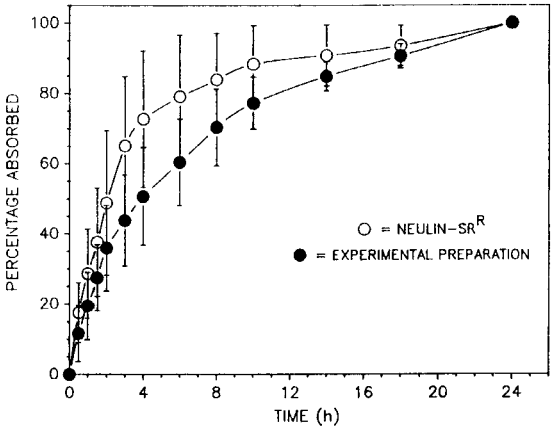

Figure 2. Mean in vivo theophylline absorption versus time profiles of Neulin-SR and the experimental preparation. Mean ± *SD*; $N = 12$.

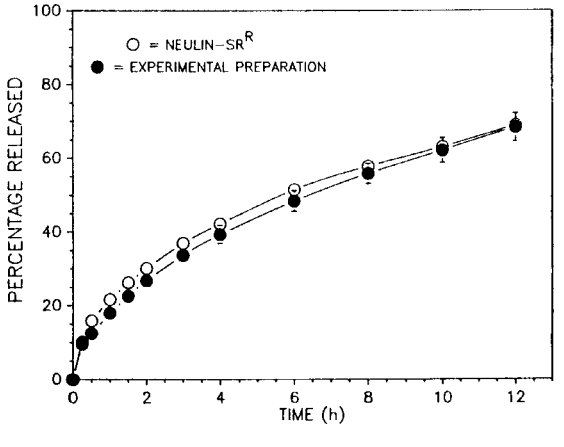

Figure 3. Mean in vitro theophylline dissolution versus time profiles of Neulin-SR and the experimental preparation. Mean ± *SD*; $N = 12$.

Neulin-SR and the experimental preparation were 2.8 hr (*SD*, 1.8 hr) and 4.2 hr (*SD*, 1.9 hr), respectively. A statistically significant difference was obtained between the $T_{50\%}$ values ($p = 0.0164$, $p < 0.05$) of the two preparations, which is consistent with the earlier analysis of the T_{max} values. However, in spite of the difference observed in the in vivo absorption, the in vitro dissolution profiles of the two preparations were almost similar. Hence, the in vitro dissolution test used may not be sufficiently sensitive to detect the difference in the in vivo performance between the two preparations.

When the ratio of the mean $T_{50\%}$ in vivo absorption over the mean $T_{50\%}$ in vitro dissolution was computed for the two preparations, a mean value of 0.7 was obtained, indicating that the rate of in vivo absorption was faster compared with the in vitro release. It is interesting to note that Benedikt et al. (13) and Yuen et al. (4) obtained a value of approximately 2.5 and 1.4, respectively, in their comparison of the in vivo and in vitro data, indicating that the in vivo process was relatively slower than that in vitro. In all the studies, the same in vitro dissolution test apparatus, namely the paddle method, was used. The inconsistency could be attributed either to the different drug release mechanism of the preparations used in the studies (as discussed previously) or to the different methods employed in analyzing the in vivo data. In our study, the Wagner–Nelson method (3) was employed to calculate the in vivo absorption versus time profile. In comparison, both the Benedikt et al. (13) and Yuen et al. (4) studies were conducted with reference to an oral solution of the drug, and a deconvolution method was applied to calculate the in vivo drug release rather than the absorption. Thus, the difference in the in vivo process used for interrelating the in vivo and in vitro data could have contributed to the discrepancy observed.

The plots of the time for in vivo absorption versus the time for in vitro dissolution of the two preparations are shown in Fig. 4. Referring to the figure, it is apparent that the two plots are divergent in nature. This is attributed to the slower rate of absorption observed with the experimental preparation compared with that of Neulin-SR, even though the in vitro dissolution profiles of the two products were almost superimposible. Notwithstanding this, a satisfactory correlation was observed between the in vivo absorption and in vitro dissolution for both preparations. Both plots in Fig. 4 appear to be relatively linear. The values of the correlation coefficient were calculated to be 0.9750 ($p < 0.001$) for Neulin-SR and 0.9969 ($p < 0.001$) for the experimental preparation.

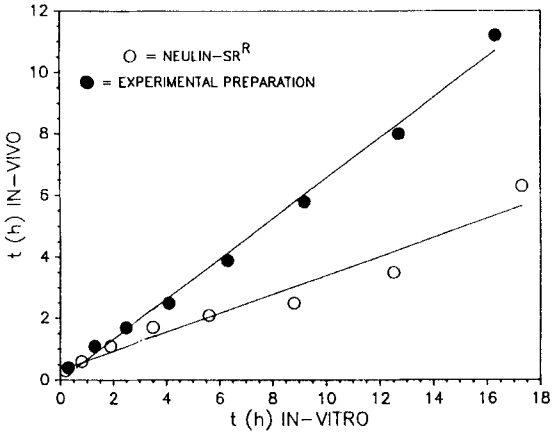

Figure 4. In vivo theophylline absorption versus in vitro dissolution times profiles of Neulin-SR and the experimental preparation.

CONCLUSION

In summary, the experimental preparation was found to be comparable to Neulin-SR in terms of the extent of absorption. However, its rate of absorption appeared to be more sustained, resulting in a relatively more uniform serum concentration profile of the drug. A satisfactory correlation was also observed between the in vitro dissolution and in vivo absorption data. In addition, the pharmacokinetic parameter values obtained are comparable to those reported in the literature for healthy, nonsmoking adults.

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