In Vivo/in Vitro Correlation of Experimental Sustained-Release Theophylline Formulations

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A novel multiparticulate sustained-release theophylline formulation, which consisted of spherical drug pellets coated with a rate-controlling membrane, was evaluated *in vivo*. Two preparations that differ solely in the coat thickness, and hence rate of *in vitro* drug release, were studied in comparison with a solution of the drug. Both preparations produced serum concentration profiles that are reflective of a slow and sustained rate of absorption. The *in vivo* release versus time profiles calculated using a deconvolution procedure showed that the two preparations differed in the rate but not the extent of drug release. Satisfactory correlation was also obtained between the *in vivo* and the *in vitro* results. When the two preparations were further compared using the parameters, time to reach peak concentration (T_p), peak concentration (C_p), and total area under the serum concentration versus time curves (AUC), a statistically significant difference was observed in the T_p and C_p values but not the AUC values, suggesting that the preparations differed in the rate but not the extent of absorption. In addition, the extent of absorption from both preparations was comparable to that obtained with the drug solution.

KEY WORDS: theophylline; sustained release; bioavailability; deconvolution; *in vivo/in vitro* correlation.

INTRODUCTION

In vitro dissolution testing enables the rapid evaluation (development) of oral sustained-release preparations. However, such *in vitro* testing cannot always predict the *in vivo* performance. Validation of the final product can be accomplished only by *in vivo* testing, ultimately in human subjects (1).

A qualitative and quantitative comparison of the plasma or serum drug concentration profile with that of a reference preparation, such as a solution, will establish the sustained-release characteristics of the dosage form. A standard procedure is to assess both the rate and the extent of absorption. Various mathematical techniques have been used in this respect and are well reviewed (2). The results derived from such analysis, particularly those that can describe the *in vivo* release or absorption versus time profile (3), are both informative and useful for interrelating *in vitro* and *in vivo* data.

The present study was conducted to evaluate the *in vivo* performance of a multiparticulate sustained-release theophylline formulation which had shown satisfactory pH-independent drug release (4). The formulation comprised spherical drug pellets coated with a mixture of ethylcellulose–methylcellulose to control the drug release. Two products of different coat thickness, which had a weight gain of 4.1% (Formula A) and 2.3% (Formula B) of coating, respectively, were chosen for the study. These produced different *in vitro* rates of drug release (Fig. 1). Their *in vivo* performance was evaluated and compared with an equivalent dose of the drug administered as a solution.

MATERIALS AND METHODS

Study Protocol

After providing written informed consent, 12 healthy, nonsmoking male volunteers between 22 and 38 years of age and weighing from 50 to 70 kg participated in this study. None were taking any medication or had a history of gastrointestinal disorders. The volunteers were randomly divided into two groups and administered the preparations according to the sequence shown in Table I. The dose of all the preparations was equivalent to 250 mg theophylline and the washout period between the treatments was 7 days. A weight of each preparation equivalent to a 250-mg dose was measured and administered in a size 0 hard gelatin capsule (Elanco Qualicaps, Lilly Industries, England), while the volume of the solution was 100 mL (2.5 mg/mL). In each case, the preparation was administered after an overnight fast with 200 mL of water for Formula A or B and 100 mL in the case of the solution. No food or drink was allowed for at least 2 hr after dosing. Lunch and supper, comprising chicken with rice, were served at 200 and 560 min, respectively. Ingestion of alcohol and xanthine-containing food or beverages was prohibited for 24 hr before, during, and 36 hr after each drug dosing. Blood samples of 5-mL volume were collected in plain vacutainers at 0 (before dosing), 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 14, 18, 24, and 36 hr after dosing for Formulas A and B and at 0 min, 10 min, 20 min, 40 min, and 1, 2, 3, 4, 6, 8, 10, 14, 18, and 24 hr after dosing for the solution. An indwelling cannula was used for drawing the blood during the first 24 hr. The subsequent sample was taken by direct venipuncture. The blood samples were allowed to stand for 2 hr before centrifuging for 10 min at 2000*g*. The serum was then transferred to separate glass containers and kept frozen until analysis.

Analysis of Serum Theophylline Concentration

Serum concentrations of theophylline were measured using a reversed-phase high-performance liquid chromatographic (HPLC) method. The HPLC system consisted of a Gilson Model 802 manometric module, a Gilson Holochrome variable-wavelength detector, and a Gilson Model 302 solvent delivery system, equipped with a Shimadzu C-R6A Chromatopac integrator. The column used was a 125 × 4-mm stainless-steel cylinder packed with 5-μm-particle size Lichrosorb RP-18 (Merck) and fitted with a direct-connect refillable guard column. The mobile phase comprised 1.5% tetrahydrofuran (THF) in 0.01 *M* sodium acetate buffer ad-

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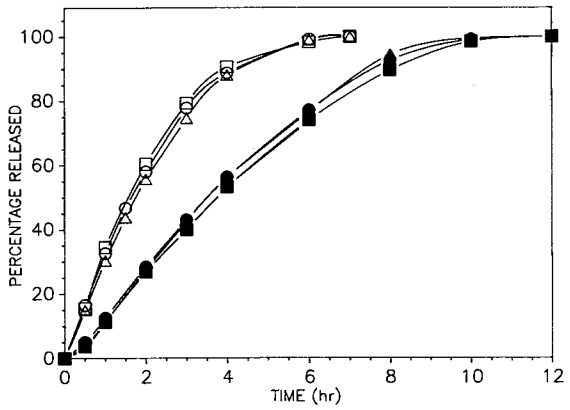

Fig. 1. *In vitro* theophylline release versus time from Formula A (filled symbols) and Formula B (open symbols) as a function of pH. (▲) pH 1; (■) pH 4; (●) pH 7.

justed to pH 4.3 with glacial acetic acid. Analysis was run at a flow rate of 1.3 mL/min with the detector operating at 273 nm.

Prior to analysis, the drug was extracted from the serum using the following procedure. A 250-μL aliquot of serum sample was accurately measured into an Eppendorf microcentrifuge tube, followed by the addition of 50 μL of 2.5 mg/100 mL β-hydroxyethyltheophylline (BHET) internal standard solution and 1 mL of 8:2 chloroform–isopropyl alcohol extracting solvent. The mixture was vortexed for 1 min and then centrifuged at 9800*g* for 1.5 min. A 0.8-mL volume of the supernatant was then removed and dried under a gentle stream of nitrogen at 60°C in a reactivial. The residue was reconstituted with 100 μL of mobile phase and a 20-μL volume was injected onto the column. All the samples were analyzed in duplicate and the average value calculated.

Theophylline standards were prepared by spiking drug-free serum in a concentration range of 1–8 μg/mL. Standard curve, recovery, and precision studies were performed using these serum standards. The recovery value for theophylline was 94.7% at 1.0 μg/mL, 93.5% at 4.0 μg/mL, and 94.8% at 8.0 μg/mL, while that of the internal standard (BHET) was 93.1%. The within-day coefficient of variation was found to be 3.6% at 2.0 μg/mL, 2.5% at 4.0 μg/mL, and 3.8% at 8.0 μg/mL. For between-day assay, the coefficient of variation values for the three concentrations were 4.3, 4.8, and 7.1%, respectively. In addition, the detector response determined with theophylline standards prepared in water in a concentration range of 0.5–16 μg/mL was found to be linear. The related xanthines, theobromine, p-xanthine, and caffeine, when injected onto the column under the same analytical conditions, were well separated from the chromatographic peaks of interest. Under the conditions of the assay method described above, the detection limit for theophylline was approximately 0.1 μg/mL per sample.

Table I. Sequence of Preparations in the Two Groups

Group (6 volunteers/group)	Period		
	I	II	III
1	Formula A	Formula B	Solution
2	Formula B	Formula A	Solution

Data Analysis

A model-independent numerical deconvolution technique based on the trapezoidal formula (3) was used to estimate the *in vivo* dissolution profiles of the two preparations. The deconvolution technique was applied to the serum concentration–time data of individual volunteers, and the results averaged to obtain the mean *in vivo* dissolution profile of each preparation. This numerical algorithm requires that the concentration data are entered on each occasion for a set of regular time points which are invariant. Whenever necessary, experimentally measured values of concentration time data were interpolated using the Spline and Akima methods of Fried and Zeitz (5). A simple computer program suitable for effecting the interpolation and deconvolution procedures was written and used in the calculation. In cases where computational instability was encountered, smoothing of the poor data points (which caused computational instability) was carried out in either the weighting data alone or both the weighting and the response data (3). This was done by substituting the serum concentration values of the poor data points, with values at the corresponding sampling times obtained from a smooth plot of the raw data.

Correlation between *in vivo* and *in vitro* results was determined by plotting the mean percentage released *in vivo* at time t versus the mean percentage released *in vitro* at time t/k, where k is an intensity factor calculated from the ratio of the mean *in vivo* over the mean *in vitro* time for 50% ($T_{50\%}$) of the dose to be released (6). The k value was calculated for both Formula A and Formula B, and the average used in the construction of the plots. In addition, correlation was also undertaken between the *in vivo* and the *in vitro* dissolution times for 10, 20, 30, 40, 50, 60, 70, 80, and 90% of drug release. The values were estimated from the mean *in vivo* and *in vitro* dissolution curves of each preparation.

Individual values of the total area under the serum concentration/time curve (AUC), peak serum concentration (C_p), and time to reach peak concentration (T_p) were also estimated from the serum concentration versus time profiles of the preparations for each volunteer. The first parameter was determined using the trapezoidal formula, while C_p and T_p were obtained directly from the serum concentration–time data (7). Whenever necessary the T_p values were corrected for a lag time of absorption which was estimated from the individual serum concentration–time curves by extrapolating the initial ascending portion of the curves to the time axis.

RESULTS AND DISCUSSION

The average serum concentration versus time profiles are presented in Fig. 2. A qualitative difference is clearly evident from the plots. Theophylline administered as a solution is rapidly absorbed achieving a peak concentration within an hour after dosing. In contrast, Formula A and Formula B both exhibit serum profiles that are reflective of a slow and sustained rate of absorption. Peak concentrations are achieved at approximately 10 and 7 hr, respectively, sug-

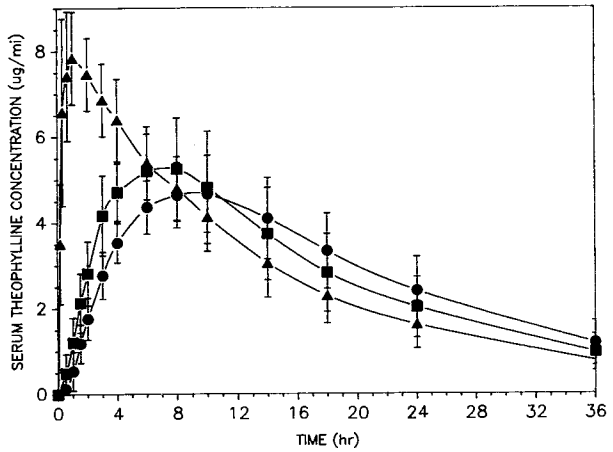

Fig. 2. Mean serum theophylline concentration versus time profiles after administration of theophylline solution (▲), Formula A (●), and Formula B (■). Mean ± SD; $N = 12$.

gesting that the absorption rate of Formula A is relatively slower.

The *in vivo* release profiles of the two preparations calculated using the deconvolution procedure are plotted in Fig. 3. It is apparent from the plots that both preparations show satisfactory *in vivo* sustained-release behavior extending over more than 14 hr in the case of Formula B and 18 hr in Formula A. While the release rate of Formula A is slower than that of Formula B, both preparations achieve essentially complete dissolution.

After correcting for lag times where appropriate, individual values of the time for 50% ($T_{50\%}$) of the dose to be released and the total fraction of dose released were calculated for each preparation. The mean $T_{50\%}$ value for Formula A is 3.6 hr (SD, 0.7 hr) and that for Formula B is 2.3 hr (SD, 0.6 hr), while the total fractions released from the two preparations are 0.99 (SD, 0.10) and 0.97 (SD, 0.10), respectively. These two parameters were used to compare the respective rate and extent of release between the two preparations. The results were subjected to analyses of variance (ANOVA) distinguishing effects due to subject, period, formulation, and residue (8), which is in accordance to the crossover part of periods I and II in the experimental design. A statistically significant difference ($P < 0.001$) was observed between the $T_{50\%}$ values of the two preparations but not those of the total fraction released ($P = 0.499$). In both cases the period effects were not statistically significant ($P > 0.05$).

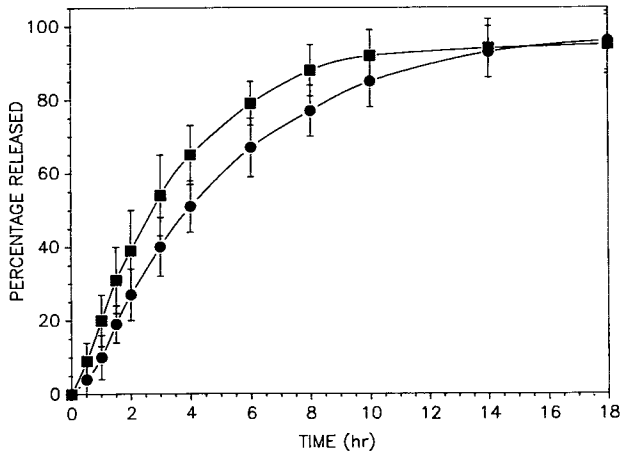

Fig. 3. Mean *in vivo* theophylline release versus time from Formula A (●) and Formula B (■). Mean ± SD; $N = 12$.

Inspection of the plots shown in Figs. 2 and 3 reveals an association between the *in vitro* and the *in vivo* results. Formula A, which has a slower *in vitro* dissolution, also exhibited a slower *in vivo* release. Therefore the results of the *in vitro* test can be used to compare the *in vivo* performance of these preparations. It is also noted that the *in vivo* release is, on the whole, slower than that achieved *in vitro*. For the two preparations, the ratio $T_{50\%}$ *in vivo* dissolution/$T_{50\%}$ *in vitro* dissolution has an average value of approximately 1.4. This is qualitatively in accord with the findings of Beckett *et al.* (9) and Benedikt *et al.* (10).

A number of approaches have been used for interrelating *in vivo* and *in vitro* data. Some of these try to relate single-point measurements, such as area under the blood concentration curve values, peak concentrations, or peak times, to the *in vitro* times for 50% drug release of other *in vitro* parameters (1). However, considering the amount of information contained in these time functions and the complexity of the processes involved, this kind of correlation is considered rather rudimentary (3). Such univariate single-point representations can obscure much of the information otherwise available from dissolution and blood level profiles. Methods based on statistical moments analysis have also been used (11–13), but likewise, they do not provide information regarding the time course of the drug release or absorption (14). By comparison, the deconvolution method used in the present study permits the time course of these processes to be estimated. An interesting feature of this method is that, depending on whether solution data or intravenous data are used as the weighting function, the *in vivo* release is calculated alone or as a combined process with absorption. In the latter case, the results of the analysis will be similar to those obtained using the Wagner–Nelson (15) or Loo–Riegelman (16) methods for the one- and two-compartment pharmacokinetic models, respectively (17).

The numerical deconvolution method used in the present study is noncompartmental in nature and makes no prior assumption of the input kinetics. The only assumption made is the linear and time-invariant behavior of the system. The algorithm has been shown to be accurate and does not create error during calculation (14). However, a disadvantage with the algorithm is its inherent tendency toward instability when the quality of the raw data is poor. Smoothing or fitting of the raw data was suggested for overcoming this problem (3). Computational instability was encountered with some of the data sets in the present study but was readily resolved by smoothing some points in either the weighting data alone or both the weighting and the response data.

The results in Fig. 4 show the plots of the percentage released *in vivo* at time t versus the percentage released *in vitro* at time t/k. The advantage of using the factor k is in obtaining a slope of near-unity, so that both *in vivo*/*in vitro* release profiles will be equally represented in the plots. It is shown in Fig. 4 that the plots of the two preparations are not coincident. This may be attributed to the *in vitro* test being

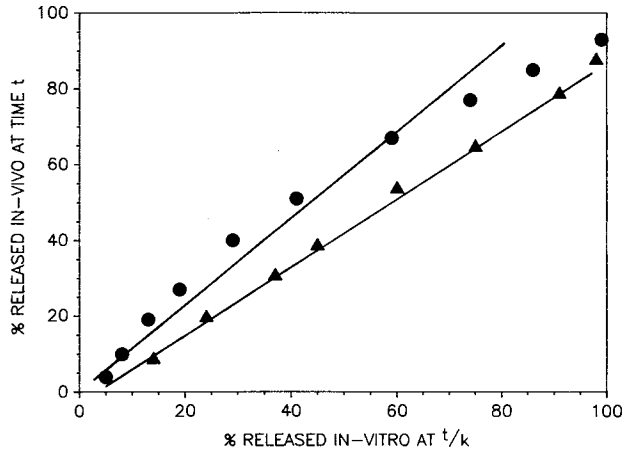

Fig. 4. Plot of mean percentage released *in vivo* at time t versus mean percentage released *in vitro* at time t/k for Formula A (●) and Formula B (▲).

slightly oversensitive in detecting a relative difference between the release rates of the two preparations and that observed *in vivo*. Nevertheless, a good relationship appears to exist between the *in vivo* and the *in vitro* dissolution rates, albeit the plot for Formula A deviates slightly from linearity. The correlation coefficient for the plot of Formula A is 0.9980 ($P < 0.001$) and that for B is 0.9991 ($P < 0.001$).

Figure 5 shows the plots relating the *in vivo* and *in vitro* dissolution times. Again, the slope of the plots appears different, but the *in vivo/in vitro* relationship is fairly linear over a majority of the points, representing about 70% of drug release. The correlation coefficients are 0.9842 ($P < 0.001$) and 0.9930 ($P < 0.001$) for Formulas A and B, respectively. The deviation of the last two points in each case is merely a reflection of the more rapid decline in the *in vivo* dissolution rates after about 60–70% of the dose have been released, compared to the *in vitro* dissolution. Presumably at this stage, the preparations have reached some parts of the gastrointestinal tract such as the cecum, where changes in agitation intensity or other local environmental factors caused the rate of dissolution to decline more rapidly. Such factors are not accounted for in the *in vitro* dissolution test methodology. Despite the shortcomings, however, the results above show that a fairly good association can be established between the *in vivo* and the *in vitro* results.

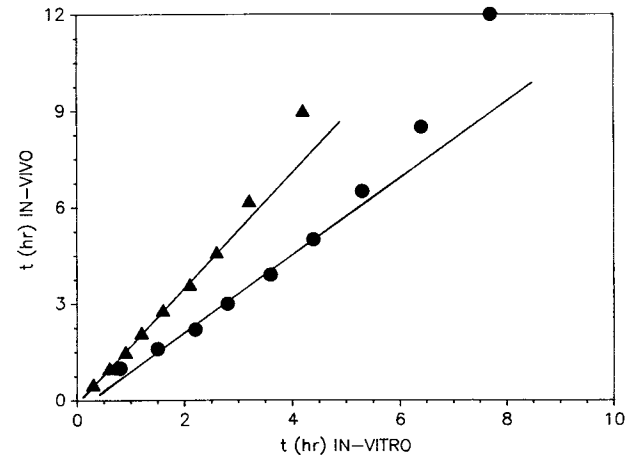

Fig. 5. Plot of *in vivo* versus *in vitro* release times for various percentages released from Formula A (●) and Formula B (▲).

The relative rate and extent of absorption of Formula A and Formula B were further compared using the parameters T_p, C_p, and AUC. The mean values of the three parameters for Formula A are 8.8 hr (SD, 1.7 hr), 4.82 μg/mL (SD, 0.86 μg/mL), and 120.5 hr μg mL^{-1} (SD, 30.7 hr μg mL^{-1}), respectively, and those for Formula B are 6.6 hr (SD, 1.5 hr), 5.44 μg/mL (SD, 109 μg/mL), and 116.9 hr μg mL^{-1} (SD, 31.0 hr μg mL^{-1}). When analyzed statistically using the ANOVA procedure mentioned earlier, a statistical difference was obtained between the values of the T_p ($P = 0.001$) and C_p ($P < 0.001$) but not those of the AUC ($P = 0.305$). The period effects were not statistically significant in the analyses of all three parameters ($P > 0.05$). In addition, the 95% confidence interval for the median ratio of the AUC values of Formula A over those of Formula B was calculated to lie between 0.95 and 1.13 and is, therefore, within the bioequivalence interval of 0.8 to 1.25.

Since T_p and AUC are related to the respective rate and extent of absorption, while C_p is related to both processes (18), these findings are consistent with the statistical results obtained with the parameters $T_{50\%}$ and total fraction released, derived from the deconvolution procedure. Therefore it can be concluded that Formula A and Formula B possess a comparable extent of *in vivo* release/absorption but differ in the rate of these processes, with Formula A being slower.

While an analysis using these parameters yielded results similar to those of the deconvolution procedure, they do not provide information regarding the *in vivo* dissolution/absorption profiles of the preparations. Thus, particularly in formulation development, analysis using the deconvolution procedure is more informative and permits *in vitro* and *in vivo* results to be interrelated.

The AUC values of the two preparations were further compared with the values obtained from the solution data. A mean of 102.0% (SD, 14.5%) was obtained for Formula A and 99.0% (SD, 12.1%) for Formula B, indicating that both preparations attained complete drug availability since theophylline administered as a solution is completely absorbed (19,20).

On the basis of the results obtained from this study, it can be concluded that the sustained-release theophylline preparations possess satisfactory *in vivo* performance. The *in vivo* drug release, particularly that of Formula A, is adequately sustained producing a satisfactory serum concentration profile over 24 hr. The contained drug is completely released and absorbed. In addition, the *in vivo* drug release rate can be effectively varied by manipulating the thickness of the film coat, which is also reflected in the results of the *in vitro* studies. In this regard, a good association can be established between the *in vivo* and the *in vitro* data, and hence the *in vitro* test could be used as a development or quality control procedure for product development.

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