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RESEARCH PAPER

Relating In Vitro/In Vivo Data of Two Controlled-Release Metformin Formulations

Kah Hay Yuen,* Kok Khiang Peh, and Boon Li Tan

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

ABSTRACT

This study was conducted to compare the bioavailability of two controlled-release metformin preparations (Diabetmin Retard and Glucophage Retard) and also to correlate the in vitro and in vivo data obtained with the two preparations. Twelve healthy volunteers participated in the study, conducted according to a completely randomized, two-way crossover design. The preparations were compared using area under the plasma concentration–time curve $AUC_{0-\infty}$*, time to reach peak plasma concentration* T_{max}*, and peak plasma concentration* C_{max}*, while correlation was determined between in vitro release and in vivo absorption. Diabetmin Retard demonstrated a slower rate of in vitro release, but a faster rate of in vivo absorption than Glucophage Retard. However, the in vivo absorption of both products was found to be slower than that of drug released in vitro. A satisfactory relationship could be established between the in vitro and in vivo results, but there was no rank order correlation. No statistically significant difference was observed between the two preparations in the parameters* $AUC_{0-\infty}$ *and* C_{max}*. However, a slight but statistically significant difference was observed between the* T_{max} *values, but it may not be therapeutically significant. Moreover, the 90% confidence interval for the ratio of the logarithmic transformed* $AUC_{0-\infty}$ *values, as well as the logarithmic transformed* C_{max} *values, of Diabetmin Retard over those of Glucophage Retard was within the acceptance criteria of 0.80–1.25.*

* To whom correspondence should be addressed. Telephone: 04-6569040. Fax: 04-6596517. E-mail: khyuen@usm.my

INTRODUCTION

In vitro dissolution testing provides an easy and convenient means to evaluate the performance of pharmaceutical preparations during their developmental stage. Formulation variables that could affect drug dissolution can be examined, leading to formulation of preparations with desired in vitro release characteristics. However, satisfactory in vitro release characteristics may not necessarily be a reliable index to predict the in vivo performance accurately. In order to validate the in vivo performance of the preparations, it is essential to test the preparations on human volunteers. Studies using human volunteers, however, are costly and tedious. Furthermore, it is not pragmatic and economical to conduct human studies on each and every batch of similar preparations.

As such, in vitro evaluations still play an important role as a quality control tool for routine screening of batch-to-batch preparations. Attempts have frequently been made to correlate in vitro dissolution data with the in vivo results obtained from human studies. Once correlation can be established between the in vitro and in vivo results, the in vitro data can then be used reliably to monitor and validate the in vivo performance of different batches of preparations without performing human studies.

In this study, an attempt was made to examine the relationship between in vitro and in vivo data obtained with two controlled-release metformin HCl tablets, Diabetmin Retard and Glucophage Retard, the former being a generic preparation. A comparison was also made of the bioavailability of the preparations.

MATERIALS AND METHODS

Products Studied

Diabetmin Retard tablets (850 mg metformin HCl) (batch no. PD 2530D; manufacturing date February 1997) was produced by Ho Yan Hor, Ipoh, Malaysia. Glucophage Retard tablets (850 mg metformin HCl) (batch no. 3158; manufacturing date July 1996; expiration date July 2000) was produced by Lipha Sante, Lyon, France.

In Vitro Dissolution Studies

The rate of metformin release from Diabetmin Retard and Glucophage Retard tablets was evaluated using USP 23 dissolution testing apparatus 1 (basket method). The dissolution tester was operated using 900 ml of dissolution medium maintained at 37°C ± 0.5°C and a rotating speed of 100 rpm. Different dissolution media were used: 0.1 M HCL and phosphate buffer BP pH 4 and pH 6.8.

Study Design

The study followed the tenets of the Declaration of Helsinki promulgated in 1964, and the protocol was approved by an ethics committee on bioavailability studies. Twelve healthy adult male volunteers between 32 and 45 years old (mean = 39 years, SD = 5 years), with height between 160 and 175 cm (mean = 166 cm, SD = 4 cm) and weighing from 56 to 75 kg (mean = 67 kg, SD = 7 kg), participated in the study after providing written informed consent. The volunteers were judged to be healthy and were not receiving any medication during the study period. Volunteers were given information on the drug and nature of the study in advance of the trial.

The volunteers were confined to the medical ward 1 day before the commencement of the clinical trial. The study was conducted according to a single-dose, two-period, two-way crossover design with six subjects in each of the two treatment groups, and a washout period of 1 week between the two phases of the study. The volunteers were randomly selected to receive one tablet of Glucophage Retard or Diabetmin Retard in the morning after a 12-hr overnight fast with 150 ml of syrup. Food was withheld for at least 2 hr after drug administration, and 100 ml of syrup water was given every half hour or ad libitum prior to lunch. Lunch and dinner of chicken with rice were served at 4 and 9 hr after drug dosing. Blood samples of 5 ml volume were collected in vacutainers (containing sodium heparin as an anticoagulant) at 0 (predose), 20 min, 40 min, and 1, 2, 3, 4, 6, 8, 10, 14, 18, and 24 hr after dosing via an in-dwelling cannula placed in the forearm. The 30-hr blood sample was taken by direct venipuncture. The blood samples were centrifuged at 2000 G for 20 min, and the plasma was transferred to separate glass containers to be kept frozen at −30°C until analysis.

Analysis of Metformin Concentration

Analysis was performed using a reversed-phase high-performance liquid chromatographic method described by Yuen and Peh (1).

Data Analysis

The in vivo absorption profiles of metformin from Glucophage Retard and Diabetmin Retard were calculated from the individual plasma-concentration-versus-

time data using the Wagner-Nelson method (2). Correlation between in vivo drug absorption and in vitro release was then determined by plotting the mean percentage absorbed in vivo at times 0.5, 1.0, 1.5, 2.0, 2.5, and 3 hr versus the mean percentage released in vitro at the same time points. Correlation was also determined between the in vivo time for 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, and 90% of drug absorbed and the corresponding time for the same percentages of drug released in vitro. The values were estimated from the mean in vivo absorption and the mean in vitro dissolution curves of each product.

Individual values of the total area under the plasma concentration–time curve $AUC_{0-\infty}$, peak plasma concentration C_{max}, and time to reach peak plasma concentration T_{max} were also estimated from the plasma concentration data of the preparations for each volunteer. The C_{max} and T_{max} values were obtained directly from the measurable plasma concentration data (3). 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 formula and the latter by dividing the last measurable plasma drug concentration with the elimination rate constant k_e. In all cases, the $AUC_{t-\infty}$ was found to be less than 15% of the $AUC_{0-\infty}$. The k_e was estimated from the terminal slope of the individual plasma concentration–time curves after logarithmic transformation of the plasma concentration values and application of linear regression (4). In addition, the time for 50% of the drug to be absorbed $T_{50\%}$ was also estimated from the individual absorption-versus-time profiles.

Statistical Analysis

All the results are expressed as mean ± standard deviation (SD). The values of $T_{50\%}$, C_{max}, $AUC_{0-\infty}$, and k_e obtained with the two preparations were analyzed using an analysis of variance (ANOVA) procedure that distinguishes effects due to subjects/group, group, period, and treatment (5). The $AUC_{0-\infty}$ and C_{max} values were logarithmically transformed prior to statistical analysis. On the other hand, the T_{max} values were compared using the Wilcoxon signed-rank test for paired samples. A statistically significant difference was considered at $p < .05$.

RESULTS AND DISCUSSION

The mean plasma metformin concentration-versus-time curves of Diabetmin Retard and Glucophage Retard

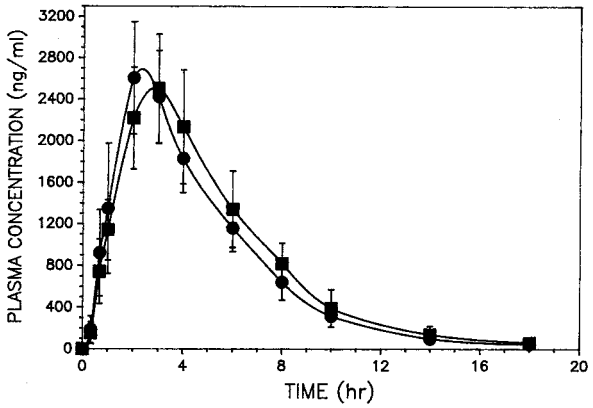

Figure 1. Mean plasma metformin concentration versus time profiles of Diabetmin Retard (●) and Glucophage Retard (■) (mean ± SD, $N = 12$).

are shown in Fig. 1. Both curves are reflective of a slow and sustained rate of drug absorption, and plasma drug concentration was still detectable at 18 hr. No lag time in absorption was observed in the plasma profiles of the two preparations. The two curves appeared to be closely similar, although Diabetmin Retard exhibited a slightly faster time to reach peak plasma concentration, leading to a higher peak plasma concentration compared to Glucophage Retard.

The mean percentage absorbed in vivo versus time profiles of Diabetmin Retard and Glucophage Retard are depicted in Fig. 2. In accord with the plasma profiles illustrated in Fig. 1, the plots in Fig. 2 showed that Diabet-

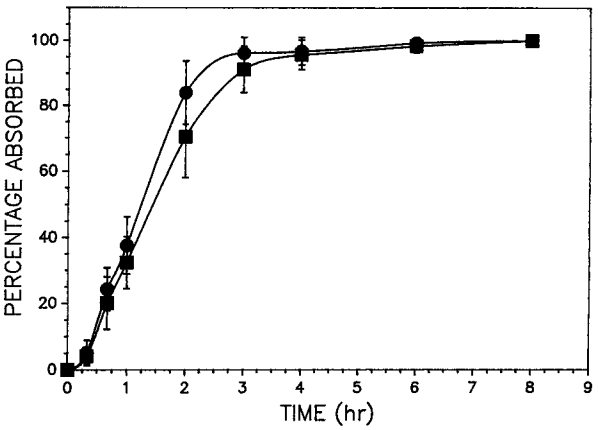

Figure 2. Mean percentage absorbed in vivo versus time profiles of Diabetmin Retard (●) and Glucophage Retard (■) (mean ± SD, $N = 12$).

min Retard achieved a slightly faster rate of in vivo absorption compared to Glucophage Retard. The mean $T_{50\%}$ values estimated for Diabetmin Retard and Glucophage Retard were 1.2 ± 0.2 hr and 1.5 ± 0.3 hr, respectively, and a statistically significant difference ($p = .0202$) was obtained between the values of the two preparations.

The in vitro drug release profiles of Diabetmin Retard and Glucophage Retard under different pH are given in Fig. 3. It can be seen that the in vitro release profiles of the two preparations were not affected by pH, and for each preparation the drug release profiles at different pH were almost superimposable. The drug release profiles of Diabetmin Retard appeared to be slower than those of Glucophage Retard. However, from the in vivo absorption profiles obtained for the two preparations shown in Fig. 2, Diabetmin Retard appeared to have a faster rate of in vivo drug absorption compared to Glucophage Retard, indicating that there was no rank correlation. This discrepancy may be ascribed to Diabetmin Retard and Glucophage Retard having different drug release mechanisms, although the two preparations are matrix controlled-release tablets. Diabetmin Retard consisted of drug dispersed in a swellable and erodible polymer, whereas Glucophage Retard consisted of drug dispersed in a nonsoluble polymer. During dissolution, no swelling was observed with the latter.

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 2.5 hr was obtained for Glucophage Retard and 1.4 hr for Diabetmin Retard, suggesting that, in both cases, the in vivo absorption rate was slower than that of the in vitro release, consistent with the results of Beckett, Staniforth, and Raisi (6), Benedikt, Steinijans, and Dietrich, (7), and Yuen, Desmukh, and Newton (8). However, in one of our earlier studies, the rate of in vivo absorption was found to be faster than that of in vitro release (9). Thus, it appears that there is no consistency in the relative rates of the in vitro and in vivo processes.

Figure 4 shows the relationship between the time for in vivo absorption versus the time for the same percentages of drug released in vitro. Both plots appeared to be linear but divergent in nature and could be attributed to the in vitro test being oversensitive in detecting a relative difference between the in vitro release rates of the two preparations compared to that observed in vivo (8). Nevertheless, a satisfactory relationship was obtained between the in vivo and in vitro data, with a correlation coefficient value of 0.9958 ($p < 10^{-6}$) for Diabetmin Retard and 0.9982 ($p < 10^{-6}$) for Glucophage Retard. The relationships between the percentage absorbed in vivo versus the percentage released in vitro at the same time points are shown in Fig. 5. As in Fig. 4, the slope of the two plots appeared different, but the in vivo/in vitro relationship was fairly linear, except for Glucophage Retard, for which the last three points showed a marked deviation. This was attributed to a rapid decline in the rate of in vitro dissolution after 80% of the dose had been released, indicating that the in vivo absorption profile was fairly linear over time compared to that of the in vitro drug release.

Table 1 shows individual values of T_{max}, C_{max}, $AUC_{0-\infty}$, and k_e obtained with the two preparations. No statistically significant difference was observed in the logarithmic

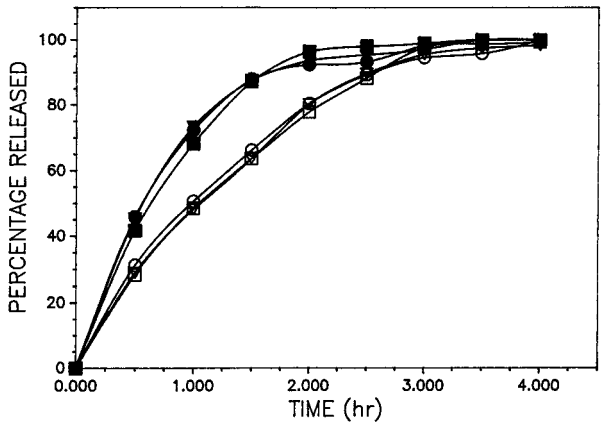

Figure 3. Percentage metformin release in vitro versus time profiles of Diabetmin Retard (open symbols) and Glucophage Retard (filled symbols) under different pH conditions: ●, pH 1; ■, pH 4; ▼, pH 6.8.

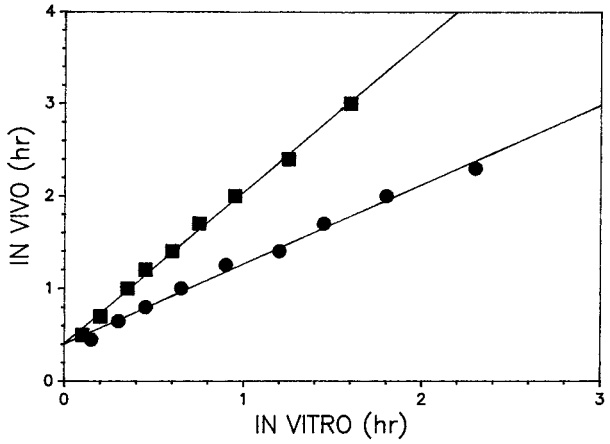

Figure 4. Plot of time for in vivo drug absorption versus time for in vitro drug released for Diabetmin Retard (●) and Glucophage Retard (■).

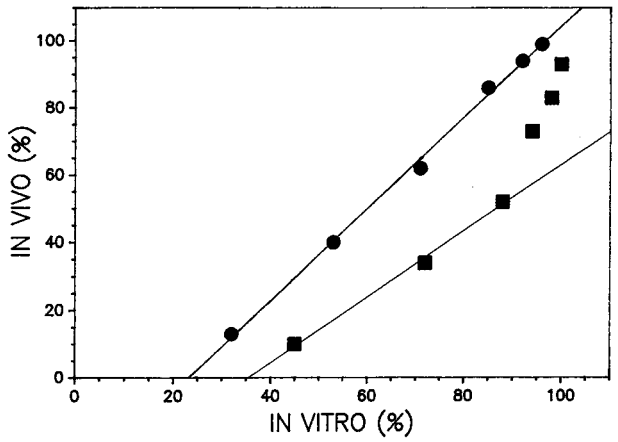

Figure 5. Plot of percentage drug absorbed in vivo versus percentage drug release in vitro for Diabetmin Retard (●) and Glucophage Retard (■).

transformed values of $AUC_{0-\infty}$ ($p = .2557$), as well as the logarithmic transformed values of C_{max} ($p = .7271$). However, a statistically significant difference was observed between the T_{max} values ($p < .05$) of the two preparations, but the slight difference may not be therapeutically important. Moreover, the 90% confidence interval for the ratio of the logarithmic transformed $AUC_{0-\infty}$ values of Diabetmin Retard over those of Glucophage Retard was calculated to lie between 0.84 and 1.04, while that of the logarithmic transformed C_{max} values was between 0.90 and 1.17, both being within the acceptable bioequivalence limit of 0.80–1.25 (10). These findings indicate that Diabetmin Retard was comparable to Glucophage Retard in the extent of bioavailability, but showed a slightly faster rate of drug absorption.

Relatively wide intersubject variation was observed in the numerical values of the pharmacokinetic parameter $AUC_{0-\infty}$, which can be attributed to differences in body weight and drug disposition among the volunteers. However, the intrasubject variability was relatively small. When estimated using the mean-square error obtained from the ANOVA analysis (11), the coefficient of variation was estimated to be 14.7%. Based on this value, a number of 12 volunteers was found to be adequate to provide a power ($1 - \beta$) of greater than 80% for detecting a statistically significant difference in AUC between the two products at a type 1 error rate (α) of 0.05 if the true difference is equal to or greater than 20% (3). In the case of the parameter C_{max}, the coefficient of variation was also relatively small, with a value of 17.5%, and the power of the test for this parameter was also found to be greater than 80%.

The k_e values of the two preparations are given in Table 1. The values of the two preparations were closely similar and not significantly different statistically ($p > .05$). Also, the values were comparable to those reported in the literature (12,13).

Table 1

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

	Glucophage Retard				Diabetmin Retard			
Subject	T_{max} (hr)	C_{max} (ng/ml)	$AUC_{0-\infty}$ (hr.ng/ml)	k_e (hr^{-1})	T_{max} (hr)	C_{max} (ng/ml)	$AUC_{0-\infty}$ (hr.ng/ml)	k_e (hr^{-1})
1	3.0	3648.9	24533.9	0.1869	2.0	2186.2	12759.4	0.2098
2	3.0	2379.3	16562.5	0.1107	3.0	2106.6	16484.0	0.1037
3	3.0	2730.2	15903.5	0.2864	3.0	2017.4	11535.3	0.3147
4	2.0	2733.8	14503.1	0.2502	2.0	2682.3	13390.4	0.2491
5	3.0	2664.2	14401.6	0.3587	2.0	2203.7	10821.3	0.3800
6	3.0	2782.7	19924.8	0.1164	3.0	3523.8	21985.2	0.1001
7	3.0	2741.3	14955.0	0.2690	2.0	4088.1	18248.7	0.3350
8	3.0	1978.3	14124.2	0.1706	3.0	2468.4	13284.4	0.2668
9	2.0	1925.4	9252.9	0.3680	2.0	2280.5	10029.0	0.3494
10	4.0	2848.5	17317.3	0.1585	2.0	3053.0	15602.1	0.2485
11	3.0	1910.0	11856.1	0.3283	2.0	3187.0	14955.3	0.3000
12	2.0	2677.5	13584.4	0.2780	2.0	2290.1	13507.8	0.3364
Mean	2.8	2585.0	15493.3	0.2401	2.3	2673.9	14383.6	0.2661
SD	0.6	488.4	3681.4	0.0900	0.5	652.6	3361.7	0.0909

CONCLUSIONS

In conclusion, a satisfactory relationship was found between the in vitro and in vivo data for both Diabetmin Retard and Glucophage Retard. For both preparations, the rate of drug absorbed in vivo was slower than that of the drug released in vitro. The in vivo absorption of Diabetmin Retard was faster than that of Glucophage Retard, while the in vitro dissolution was slower, indicating that there was no rank-order correlation. Diabetmin Retard was comparable to Glucophage Retard in the extent of bioavailability, but showed a slightly faster rate of absorption, which may not be therapeutically important. Also, the k_e values obtained in the present study were comparable to those reported in the literature.

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