ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
15 (1997) 439–445

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

In-vivo/in-vitro correlation of four extended release formulations of pseudoephedrine sulfate

Parviz Mojaverian[a,*], Jack Rosen[b], Winston A.Vadino[c], Stephen Liebowitz[c], Elaine Radwanski[a]

[a]*Department of Drug Metabolism & Pharmacokinetics, Schering-Plough Research Institute, 2015 Galloping Hill Rd., Kenilworth, NJ 07033, USA*

[b]*Department of PACRD, Schering-Plough Research Institute, 2015 Galloping Hill Rd., Kenilworth, NJ 07033, USA*

[c]*Department of Formulation R & D, Schering-Plough Research Institute, 2015 Galloping Hill Rd., Kenilworth, NJ 07033, USA*

Received for review 16 November 1995; revised manuscript received 15 March 1996

Abstract

An in-vivo/in-vitro correlation was established for four formulations of pseudoephedrine sulfate modified release tablets exhibiting different in-vivo and in-vitro release rate and absorption characteristics. In-vitro release rate data were obtained for 12 individual tablets of each formulation using the USP Apparatus 2 paddle stirrer at 50 rev min^{-1} in 1000 ml 0.1 N hydrochloric acid for the first hour followed by 0.1 M phosphate buffer at pH 7.5 for hours 2–16. Inspection of the individual and mean release rate data indicated that the in-vitro release rate of pseudopehedrine sulfate was consistent with the intended design of the four extended release formulations. The in-vivo bioavailability and pharmacokinetics of these formulations were evaluated in 20 healthy volunteers under fasted conditions. Wagner–Nelson analyses of the in-vivo data revealed extended release absorption profies for all four formulations. Linear regression analyses of the mean percentage of dose absorbed versus the mean in-vitro release resulted in statistically significant correlations ($r^2 > 0.99$, $p < 0.0001$) for each formulation. Qualitative rank order correlations were observed among all combinations of in-vivo and in-vitro parameters. These data support a Level A correlation between in-vivo absorption profiles and in-vitro release rates of four pseudoephedrine sulfate extended release formulations determined in fasted healthy volunteers.

Keywords: Absorption profile; Extended release tablet; In-vivo/in-vitro correlation; Pseudoephedrine sulfate; Release rate

1. Introduction

An oral extended release cold product containing pseudoephedrine sulfate was developed. Evaluation of a product's performance using in-vitro dissolution methodologies which can predict its

* Corresponding author. Present address: 7 Abraham Rd., Whitehouse Station, NJ 08889-3200.

PII S0731-7085(96)01834-1

in-vivo bioavailability is a powerful tool in the process of drug product development. Factors which affect a product's rate and extent of absorption include dissolution, solubility, chemical stability, protein binding, gastrointestinal transit time, metabolism and excretion. In designing an extended release product, it is the dissolution rate of the drug which is the rate-limiting process and hence sets the conditions for the rate at which the drug is absorbed and available for systemic effect.

Since performing biostudies on every manufactured batch is impractical and costly, formulators must rely on in-vitro testing to insure batch-to-batch uniformity and consistency in bioavailability. Dissolution testing is dependent on the instrument's hydrodynamic condition and the dissolution medium. It cannot predict physiological variables such as gastric emptying time and presystemic or first-pass metabolism. The in-vivo parameters that help assess the rate and extent of absorption, AUC, C_{max} and T_{max}, may not be sufficient to evaluate the pharmacokinetic performance, particularly the absorption rate of extended release formulations. However, the in-vivo and in-vitro data combined add another useful dimension for evaluation of a product's performance.

A 1:1 relationship between in-vivo absorption and in-vitro dissolution is the highest level of correlation achievable; hence the definition Level A correlation which is likely to occur when the in-vitro dissolution rate is independent of test conditions such as pH, agitation, medium, and temperature. By deconvoluting the plasma concentration–time curve using model-independent methods such as Wagner–Nelson methods or direct mathematical deconvolution [1,2] and time correction factors [3,4], it is possible to obtain reproducible correlations between in-vitro dissolution rate and in-vivo absorption profiles.

The advantage of a Level A correlation is that it provides a truly meaningful quality control prodedure which is predictive of its in-vivo performance. Additionally, a change in a manufacturing site, method of manufacture, raw material supplier, minor formulation composition, or potency strength of the same formulation of an existing dosage form without the need for additional bioquivalence studies in humans can be justified.

The purpose of this study was to establish a correlation between in-vitro release rate as measured by dissolution studies and in-vivo plasma levels for four extended release formulations of pseudoephedrine sulfate.

2. Materials and methods

Four extended release tablet formulations containing 240 mg pseudoephedrine sulfate were manufactured specifically for use in this study. Each formulation was designed to exhibit different release rates of pseudoephedrine sulfate. One of the formulations (D) was designated as the standard formulation and the other three were designed to afford relatively faster or slower pseudoephedrine sulfate release rate profiles compared to the standard formulation.

The dosage forms used in this study were based on a matrix design using a mixture of cellulosic polymers. Drug release occurs by a combination of diffusion and erosion mechanisms. Varying the ratio of the cellulosic constituents will control the release rate of the drug from the dosage form. Once the designed standard formulation was identified based on release rate and in-vivo bioavailability characteristics, additional formulations were prepared with release rates faster and slower than that of the reference standard. A similar approach was successfully used for a chlorpheniramine maleate extended release formulation [5].

2.1. In-vitro dissolution testing

Dissolution testing was performed using a USP Apparatus 2 paddle stirrer operating at 50 rev min^{-1}. Based on solubility data, two dissolution media were used: 1000 ml 0.1 N hydrochloric acid for the first hour (simulating a gastric residence time in the fasted state) followed by 0.1 M phosphate buffer at pH 7.5 for hours 2–16. Multicomponent UV–Vis spectrophotometric analysis using a Hewlett Packard 8450 photodiode array spectrophotometer was used to resolve the spectrum of pseudoephedrine sulfate. Approximately 10 ml aliquot samples were withdrawn from each

dissolution vessel at 1, 2, 4, 6, 8, 10, 12, and 16 h and filtered prior to spectrophotometric analysis. The wavelength range for the multi-component analysis was optimized after successful completion of drug linearity and recovery from the analytical placebo experiments. Replicate multi-component analyses of different concentrations of standard solutions yielded relative standard deviations of less than 0.5%.

2.2. In-vivo studies

2.2.1. Study design

Twenty normal adult male volunteers between the ages of 19 and 39 years (mean ± SD: 28 ± 6) and weighing between 138 and 190 lbs in accordance with current actuarial tables (±10%) were empaneled for this randomized, four-way crossover study. All subjects were determined to be in good health through medical history, physical examination, electrocardiogram and routine laboratory tests. Each subject signed a written informed consent prior to study participation.

Twelve hours prior to the start of each study phase, the volunteers were confined to the study area. A light snack was served on the night before drug administration after which an overnight fast was maintained. In the morning, each subject received one treatment from the four manufactured batches with 120 ml (4 fl. oz.) of tap water.

A 1-week drug-free washout period separated each of the four phases of the crossover. After dosing, fasting continued until the 4 h blood samples were obtained after which a light lunch was served. 10 ml of blood was drawn immediately prior to drug administration (0 h) and then at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36 and 48 h after dosing. The specimens were immediately centrifuged for 15 min, the plasma samples were frozen and maintained at −15°C until the time of analysis. Psuedoephedrine plasma concentrations were measured by a previously validated, specific gas–liquid chromatographic prodedure with a limit of quantitiation (LOQ) of 10 ng ml^{-1} [6].

2.3. Pharmacokinetic analysis

Plasma concentrations above the LOQ were used for pharmacokinetic analysis employing model-independent methods [7]. The maximum plasma concentration, C_{max}, and the time of C_{max}, T_{max}, were the observed values. The terminal phase rate constant, K, was calculated as the negative of the slope of the log–linear terminal portion of the plasma concentration–time curve using linear regression. The area under the plasma concentration–time curve from time zero to the time of the last quantifiable concentration time point, tf, AUC(tf) was calculated using the linear trapezoidal rule and extrapolated to infinity by the following equation:

$$\mathrm{AUC(I)} = \mathrm{AUC(tf)} + C_{tf}/K$$

where C_{tf} is the estimated concentration at tf and AUC(I) is the area under the plasma concentration–time curve from time zero to infinity.

Absorption profiles of pseudoephedrine sulfate were evaluated for each subject using the Wagner–Nelson function [1]:

$$F(t) = C(t) + K \cdot \mathrm{AUC}(t)$$

The Wagner–Nelson function was expressed as a percentage of its asymptotic value, $K \cdot \mathrm{AUC(I)}$, to yield the percentage of dose absorbed:

$$\%\ \text{dose absorbed} = \{[C(t) + k \cdot \mathrm{AUC}(t)]/K \cdot \mathrm{AUC(I)}\} \times 100$$

2.4. In-vivo/in-vitro data analysis

In this study, mean pharmacokinetic and mean release rate data were used to establish the correlations to minimize intra- and inter-subject variability. The method of Levy and Hollister [3,4] was utilized to determine in-vivo/in-vitro correlation. This method, which corrects for the in-vivo lag time (t_{lag}), utilizes the Wagner–Nelson method expressed in terms of the semilogarithmic percentage unabsorbed as a function of time. An intensity factor, I, which relates the in-vivo lagtime (t_{lag}) and the in-vitro sampling time is defined as follows:

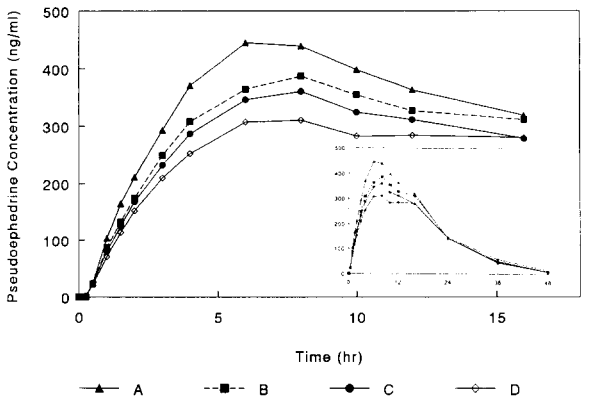

Fig. 1. Mean plasma concentration–time profiles for four pseudoephedrine sulfate extended release formulations following oral administration to 20 normal male volunteers. The inset shows the profile on an extended time axis (up to 48 h post-dose).

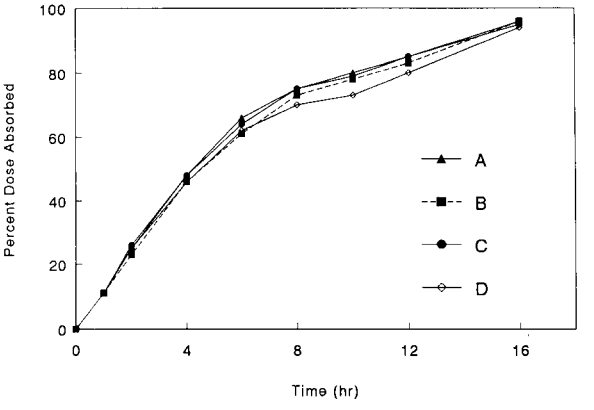

Fig. 2. Mean in-vivo absorption profiles following oral administration of four pseudoephedrine sulfate extended release formulations over a 16 h period. The percentage dose absorbed at each time point tested was calculated by the Wagner–Nelson method.

$$I = \frac{\text{Time required for 50\% absorption in vivo}}{\text{Time requried for 50\% release in vitro}}$$

$$t_{lag} = \frac{t - \text{lag time (in vivo)}}{I}$$

where t is the original in-vitro sampling time.

The percentages of the dose released at t_{lag} and I for each formulation were determined from best-fit third degree polynomial equations established for the in-vitro release profiles.

3. Results and discussion

The mean plasma pseudoephedrine concentration–time data following each of the four treatments are presented in Fig. 1. When the concentration–time profiles were examined up to 16 h post-administration, the formulations exhibited similar profiles differing only in the rate at which pseudoephedrine was released to become available for absorption (Fig. 1). The mean pharmacokinetic parameters are presented in Table 1. There was a rank order increase in both C_{max} and AUC(I) from Formulation A to Formulation C. As expected, there was an inverse relationship in T_{max} where the longest mean T_{max} was observed for Formulation A and the shortest T_{max} was observed for Formulation C. The terminal phase half-life $t_{1/2}$ ranged from 6.4–7.8 h.

The Wagner–Nelson plots of percent of dose absorbed versus time for the four extended release formulations are presented in Fig. 2. The absorption rate over a 16 h interval was similar for all sustained release formulations as reflected by the qualitatively similar profiles. The same rank order correlations were observed between the in-vivo percent dose absorbed (as shown in Fig. 2) and the

Table 1
Mean (% RSD) pharmacokinetic parameters of pseudoephedrine

Formulation	AUC(tf) ng h ml^{-1}	AUC(I) ng h ml^{-1}	C_{max} (ng ml^{-1})	T_{max} (h)	$t_{1/2}$ (h)
C	8610 (10)	8822 (20)	460.1 (13)	6.9 (22)	6.4 (15)
B	8006 (22)	8225 (21)	397.7 (15)	7.8 (22)	6.9 (14)
D (Standard)	7547 (25)	7830 (24)	366.3 (17)	7.4 (22)	7.3 (12)
A	7315 (25)	7597 (25)	334.8 (18)	9.3 (44)	7.8 (24)

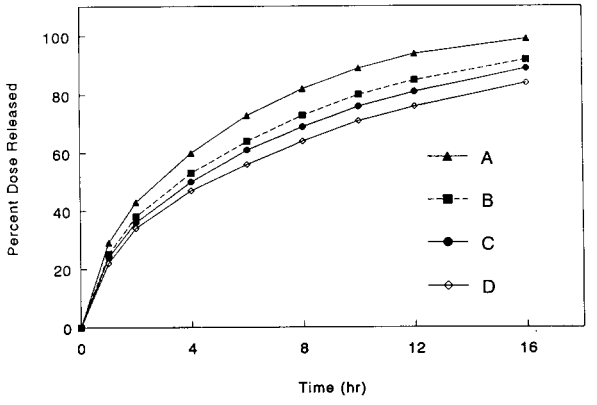

Fig. 3. Mean in-vitro release profiles for four extended release formulations of pseudoephedrine sulfate over a 16-h period.

Table 2
In-vivo/in-vitro regression analysis[a] (in-vivo percent dose absorbed (Y) versus in-vitro percent dose released (X))

Formulation	Slope (m)	Intercept (b)	Coefficient of determination (r^2)	p- value
A	1.19	−24.15	0.995	0.0001
B	1.27	−21.99	0.995	0.0001
D (Standard)	1.30	−18.89	0.992	0.0001
C	1.32	−17.16	0.988	0.0001
Mean	1.26			
% RSD	4.50			

[a] $Y = mX + b$.

in-vitro percent dose released (Fig. 3). These curves are superimposable, indicating a 1:1 relationship which defines a Level A correlation [7]. As expected, there is a discrepancy in this relationship due to in-vivo absorption lag time which is reflected by a negative y intercept (Fig. 4). The corresponding parameters describing the regression line are summarized in Table 2. There is a statistically significant ($r^2 > 0.98$; $p = 0.0001$) linear in-vivo/in-vitro correlation for all four formulations (Table 2). The slopes are similar, and

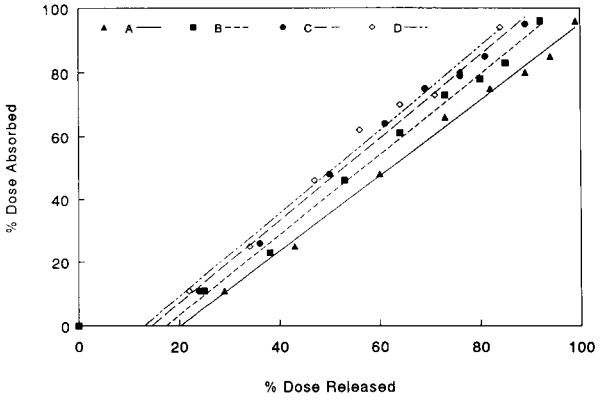

Fig. 4. Plots of mean percentage of dose absorbed versus mean percentage dose released for the extended release formulations of pseudoephedrine sulfate. The line of best fit is shown for each formulation. The corresponding regression equations are listed in Table 2.

greater than one, suggesting that the pseudoephedrine sulfate in-vivo release rate was slightly slower than its in-vitro release rate. The observed negative y intercepts were due to the absorption lag time following oral administration of enteric coated and extended release dosage forms [8–10]. The calculated lag times and intensity factors summarized in Table 3 indicate a rank order consistent with the observed in-vitro release profiles (Fig. 3).

The in-vivo/in-vitro discrepancy observed in Fig. 4 can be mathematically corrected to yield a simple linear correlation between percent of dose absorbed and percent of dose released. The percentage of dose absorbed at time t versus the adjusted percentage of dose released at t_{lag} is shown in Fig. 5. The regression analysis of these data revealed a significant linear correlation ($r^2 = 0.98$) with slope values approaching unity and the lines passing through the origin.

Table 3
Summary of lag times and intensity factors

Formulation	Lag time (min)	Intensity factor
C	19	1.55
B	30	1.36
D (Standard)	34	1.05
A	49	1.07

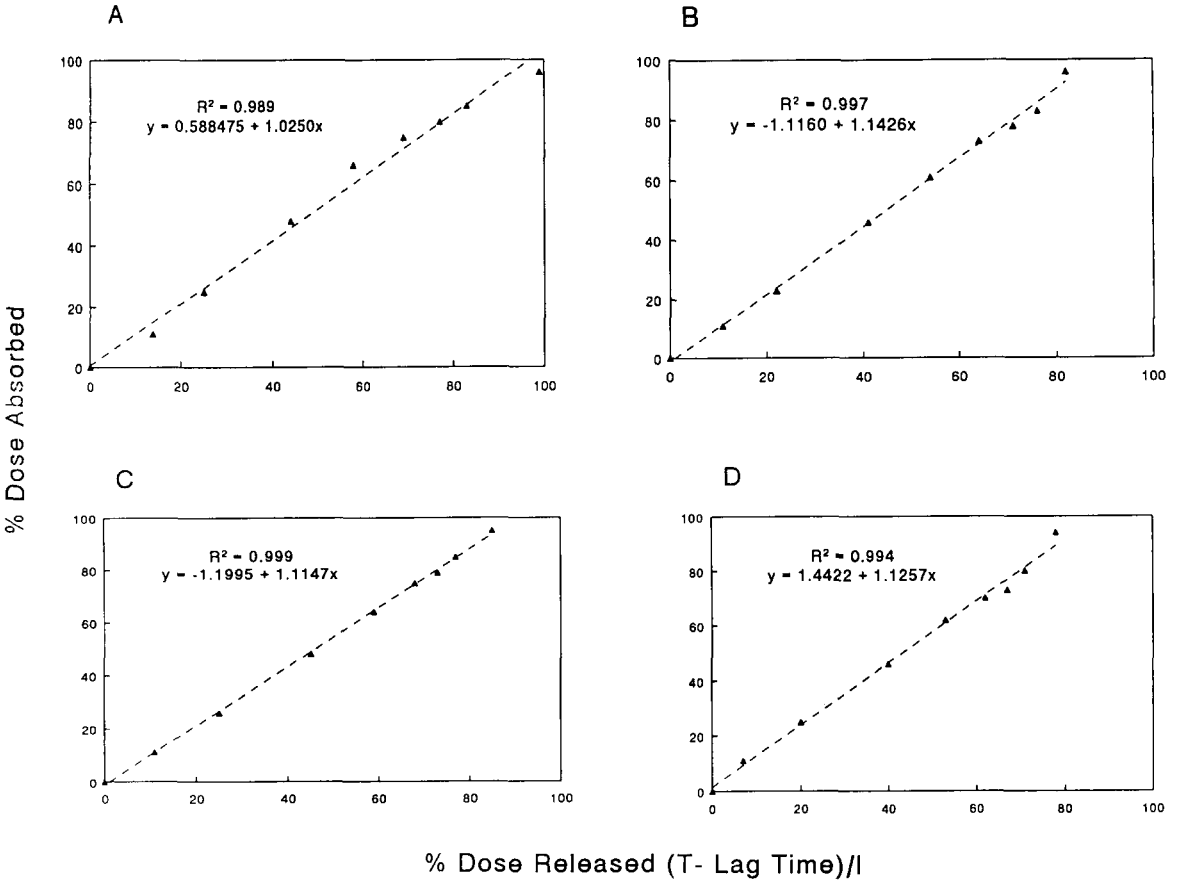

Fig. 5. Plots of mean percentage of dose absorbed versus mean percentage dose released at t_{lag} for pseudoephedrine sulfate extended release formulations. The line of best fit is presented in each case.

4. Conclusions

In-vivo/in-vitro correlations are important for demonstrating meaningful, predictive, and discriminating in-vitro dissolution specifications. The significant linear correlations between in-vitro and in-vivo parameters reported herein are consistent with Level A correlation guidelines described by the FDA/AAPS task force [11]. The dissolution technology can now serve as a tool to assure batch-to-batch drug release uniformity, to assess the impact of scaleup of batches, change in manufacturing site and minor changes in formulation and manufacturing equipment, thereby minimizing requirements for in-vivo experiments. However, to show bioequivalence of extended or controlled release products, the suitability of in-vitro dissolution tests as surrogate markers for bioequivalence has been neither established nor uniformly accepted worldwide [12].

References

[1] J. Wagner, Fundamentals of Clinical Pharmacokinetics, Drug Intelligence Publications, Hamilton, IL, 1975, pp. 173–180.
[2] N. Watari, N. Kaneniwa and M. Hanano, Int. J. Pharm., 6 (1980) 155–166.
[3] G. Levy and L. Hollister, J. Pharm. Sci., 54 (1965) 1121–1125.
[4] G. Levy and L. Hollister, J. Pharm. Sci., 53 (1964) 1446–1452.
[5] P. Mojaverian, E. Radwanski, C.C. Lin, P. Cho, W. Vadion and J. Rosen, Pharm. Res., 9 (1992) 450–455.

[6] L. Cummins and M. Fourier, Anal. Lett., 2 (1969) 403-409.
[7] M. Gibaldi and D. Perrier, Pharmacokinetics, Marcel Dekker, New York, 1982, pp. 409–417.
[8] P. Mojaverian, M. Rocci, D. Connor, W. Abrams and P. Vlasses, Clin. Pharmacol. Ther., 41 (1987) 11–17.
[9] M. Rocci, P. Mojaverian, R. Davis, R. Ferguson and P. Vlasses, Clin. Pharmacol. Ther., 42 (1987) 45–49.
[10] S. Davis, J. Controlled Rel., 2 (1985) 27–38.
[11] J. Skelly, G. Amidon, W. Barr, L. Benet, J. Carter, J. Robinson, V. Shah and A. Yacobi, J. Pharm. Sci., 79 (1990) 849–854.
[12] V. Shah and R. Williams, in A. Jackson (Ed.), Generics and Bioequivalence, CRC Press, Boca Raton, Florida, 1994, pp. 101–111.