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IN VIVO PERFORMANCE OF CONTROLLED RELEASE PELLETS OF DILTIAZEM HCL

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The test formulation of controlled release diltiazem pellets was evaluated *in vivo,* in comparison with Herbesser SR. Six healthy volunteers participated in the study, conducted according to a randomized, two-way crossover study design. The preparations were compared using the pharmacokinetic parameters plasma concentration-time curve ($AUC_{0-\infty}$), peak plasma concentration (C_{max}) and time to reach maximum plasma concentration (T_{max}) were estimated from the plasma concentration-time profiles for each volunteer. The test formulation was found to be comparable with the Herbesser SR in the extent of bioavailability but differ in the rate of absorption, the test formulation being less sustained. No lag time was observed in any of the volunteers indicating that both formulations started to release their drug content immediately upon rupture of the capsule but in sustained manner. Moreover, the values of pharmacokinetic parameters obtained were comparable to those reported in the literature.

Keywords:

INTRODUCTION

In vitro dissolution test is a useful tool during the initial development of solid oral dosage forms, especially modified-release formulations. During developmental stage, a number of variables affecting the drug dissolution on drug release can be investigated, which in turn provide the basis for formulating a product with the desired *in vitro* drug release characteristics. However, *in vitro* dissolution data cannot directly predict the *in vivo* performance of the formulated product. Therefore, it is essential for a formulation to be verified through *in vivo* testing after satisfactory *in vitro* release profile has been obtained. For this purpose, a comparative *in vivo* study is usually conducted on the new formulation against an established reference preparation. A test formulation of controlled release pellets (150g) was prepared by coating diltiazem-loaded pellets with a 7% Eudragit NE40-diltaizem mixture. Drug release of the test formulation was sufficiently sustained and independent of pH and agitation rate. Moreover, the drug release was found to be stable after storage for one year. In view of satisfactory *in vitro* release characteristics, the present study was therefore, conducted to evaluate the *in vivo* performance of test formulation using human volunteers. In this regard, an established proprietary product, Herbesser SR was used for comparison.

MATERIALS

Diltiazem hydrochloride (Sigma, USA), verapamil hydrochloride (USP), Herbesser SR Capsules 90 mg, Batch No: UN 120, Expiry date: Mar 2004 (Tanabe, Japan), acetonitrile, HPLC grade (Malinckrodt, USA), diethyl Ether, AR (BDH, England), N-hexane, AR (Malinckrodt, USA), ammonium dihydrogen ortho phosphate, AR (BDH, England), triethylamine, AR (Fluka, Switzerland) and phosphoric acid, AR (BDH, England)

METHODS

In vivo study protocol

In vivo study was conducted according to a randomized two-way crossover design. Six (6) healthy non-smoking adult male volunteers between 27 and 40 years old (Mean =34 years, SD =6 years), with heights from 158 to 174 cm (Mean =164 cm, SD =6 cm), and weighing from 49 to 72 kg (Mean =61 Kg, SD =10 Kg), participated in the study which has been approved by an ethic committee. 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. Six volunteers were randomly divided into 2 groups of 3 each and administered the preparations according to the schedule shown below:

Group	Period	
	I	II
1	Herbesser SR	Test Formulation
2	Test Formulation	Herbesser SR

In the first trial period, the volunteers in the first group were given the capsules of Herbesser SR 90 mg and the second group was given capsules containing the test formulation equivalent to 90 mg of drug. After a washout period of one week, each volunteer then received the alternate product. Both preparations were administered with 240 ml of water in the morning at 9.00 a.m. after 12 hours fast. Food and drinks were withheld for at least 2 hours after dosing. Blood samples of 7-ml volume were collected in vacutainers (containing sodium heparin as anticoagulant) at 0 (before dosing), 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14, 16, and 24 hours after dosing via an in-dwelling cannula placed in the forearm. Two more blood samples were taken at 36 and 48 hours via direct veinpuncture. The blood samples were centrifuged for 15 min at 3500 rpm and the plasma was

Table 1
Individual numerical values of AUC_{0-t}, $AUC_{0-\infty}$, C_{max} and T_{max} from Herbesser SR and test formulation

Subject	Herbesser SR				Test formulation			
	AUC_{0-t} (ng.hr/ml)	$AUC_{0-\infty}$ (ng.hr/ml)	C_{max} (ng/ml)	T_{max} (hr)	AUC_{0-t} (ng.hr/ml)	$AUC_{0-\infty}$ (ng.hr/ml)	C_{max} (ng/ml)	T_{max} (hr)
S1	1010.9	1037.1	42.0	14.0	1422.3	1461.9	68.7	10.0
S2	268.9	333.4	22.9	14.0	462.9	488.7	37.6	4.0
S3	722.3	828.0	43.4	10.0	692.4	764.3	50.7	12.0
S4	1036.1	1082.3	54.4	4.0	989.2	1022.8	66.1	6.0
S5	673.3	690.5	36.5	16.0	567.9	575.7	31.7	8.0
S6	782.7	836.3	38.7	6.0	735.9	781.6	55.1	6.0
Mean	749.0	801.3	39.7	10.7	811.8	849.1	51.7	7.7
SD	279.0	271.2	10.3	4.8	348.0	352.7	14.9	2.9

Table 2
Individual pharmacokinetic values (K_e, t ½ and V_d/f) of diltiazem from two formulations

Subject	Herbesser SR			Test formulation		
	K_e (hr^{-1})	t ½ (hr)	V_d/f (L/Kg)	K_e (hr -1)	t ½ (hr)	V_d/f (L/Kg)
S1	0.126	5.5	12.6	0.119	5.9	9.4
S2	0.114	6.1	45.7	0.181	3.8	19.6
S3	0.174	4.0	8.7	0.126	5.5	13.0
S4	0.104	6.7	16.4	0.089	7.8	20.2
S5	0.104	6.7	19.3	0.154	4.5	15.6
S6	0.116	6.0	13.3	0.162	4.3	10.2
Mean	0.123	5.8	19.3	0.138	5.3	14.7
SD	0.026	1.0	13.4	0.033	1.4	4.6

transferred to new glass tubes and kept at –20°C until analysis.

Analysis of plasma diltiazem concentration

The plasma samples were analyzed using a reversed-phase high-performance liquid chromatography (HPLC) method. The HPLC system comprised a Jasco PU-980 Intelligent HPLC pump, a Gilson 119 UV/VIS detector (Gilson Medical Electronics, Villiers-le-Bel, France) connected to a Hitachi D-2500 integrator (Hitachi, Tokyo, Japan) and a Rheodyne 7125 sample injector fitted with a 50 µl sample loop. The detector was operated using a sensitivity range of 0.005 AUFS and wavelength of 237 nm. A LiChrospher 100 RP-18e reversed phase column (5µm, 250-x 4.6 mm ID) (Merck, Germany) fitted with a refillable guard column (Upchurch Scientific, Oak Harbour, WA, USA) and packed with Perisorb RP-18; 30-40 µm pellicular stationary phase (Upchurch Scientific, Oak Harbour, WA, and USA) was used for the separation. The mobile phase comprised 0.1M ammonium dihydrogen phosphate and acetonitrile (62:38 v/v). Triethylamine (0.08%) was added before the pH was adjusted to 5.9 with 85% phosphoric acid. Analysis was run at a flow rate of 1.0 ml/min and quantified using peak height.

Prior to injection, diltiazem was extracted from the plasma samples according to the following procedure: 1.0 ml aliquot of plasma sample was measured accurately into a 10.0 ml screw capped glass tube, followed by the addition of 50 µl (4 µg/ml) verapamil HCl in aqueous solution as internal standard. After this, 4.0-ml mixture of diethyl ether and n-hexane (1:1) was then added as the extracting solvent. The mixture was vortexed for 1 min using a vortex mixer and then centrifuged (Labofuge 200, Heraeus Sepatech, Germany) at 3500 rpm for 10 minutes. The upper organic layer was transferred into a reactivial (Pierce Reacti-vial, USA) and then evaporated to dryness at 40°C under a gentle stream of nitrogen gas. The residue was reconstituted with 75 µl of mobile phase and 50 µl injected onto the column.

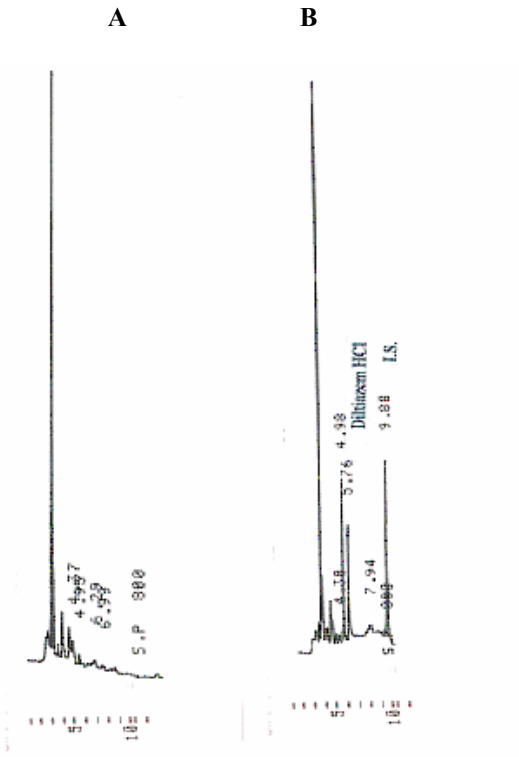

Fig.1: Chromatograms of blank plasma and sample obtained from a volunteer at 4 hours after drug administration.

The standard curve was prepared by spiking drug free plasma with a known weight of diltiazem at concentration levels of 5, 10, 20, 40, 80 and 160 ng/ml. The standard plasma samples were stored at –20°C in glass bottles.

Pharmacokinetic parameters analysis

The pharmacokinetic parameters, namely total area under the plasma concentration-time curve ($AUC_{0-\infty}$), peak plasma concentration (C_{max}) and time to reach maximum plasma concentration (T_{max}) were estimated from the plasma concentration-time profiles for each volunteer. The C_{max} and T_{max} values were obtained directly from the plasma-concentration data (Weiner, 1981). The $AUC_{0-\infty}$ was calculated by adding the area from time zero to the last sampling time (AUC_{0-t}) and the area from the last sampling time 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 apparent elimination rate constant (k_e). In all cases, the $AUC_{t-\infty}$ was found to be less than 10% 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 (Gibaldi and Perrier, 1982). On the other hand, the elimination half-life (t½) was calculated from the quotient ln $2/k_e$, while the apparent volume of distribution (Vd/f) was calculated as Dose/ ($AUC_{0-\infty}$ x k_e). The *in vivo* absorption profiles of the formulations were also calculated from the individual plasma concentration versus time data using the Wagner-Nelson method (1964).

Statistical analysis

The calculated values of the parameters, $AUC_{0-\infty}$, C_{max}, t½, k_e and Vd/f obtained with the two preparations were analyzed statistically using an analysis of variance (ANOVA) procedure which distinguished effects due to subjects, periods, and treatment (Wagner, 1975). $AUC_{0-\infty}$ and C_{max} values were logarithmically transformed prior to the analysis. On the other hand, the T_{max} values of the two preparations were analyzed using the Wilcoxon Signed Rank test for paired samples. A statistically significant difference was considered when $P < 0.05$. In addition, the 90% confidence interval for the ratio of $AUC_{0-\infty}$ as well as Cmax values of the test formulation over those of the Herbesser SR was also determined.

RESULTS AND DISCUSSION

Chromatograms obtained with blank plasma and plasma sample of a healthy volunteer after 4 hours dosing with test formulation containing 90 mg diltiazem are shown in fig.1. The retention times of diltiazem HCl and internal standard (verapamil HCl) were 5.78 and 9.88 minutes respectively. The blank sample was clean and no interfering peak was observed at the retention times of diltiazem and verapamil HCl. The mean plasma diltiazem concentration versus time profiles of Herbesser SR and the test formulation are shown in fig.2. The plasma concentration profiles of both products showed that the plasma concentrations of diltiazem were sustained and detectable even at 36 hours. No lag time in the plasma concentration of the two formulations was noted. There was gradual increase in the plasma concentration reaching a peak at approximately 4-6 hours after administration and thereafter maintained for up to about 12 hours. The test formulation had a comparatively higher plasma concentration profile, being reflective of a slightly faster rate of absorption. The double peak in the mean plasma profiles of the two formulations was also apparent in fig.2. A similar effect has also been reported following the administration of SR multiparticulate diltiazem formulation (240 mg) after an overnight fast or heavy breakfast (Wilding *et al.,* 1991). The secondary peak was ascribed to be disruption of the device, which led to the elevated peak at later time intervals or more likely that the drug was subjected to some form of enterohepatic recycling (Colburn, 1984) or interruption in the drug absorption (Funaka *et al.*, 1986).

The individual numerical values of AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, and T_{max} obtained with Herbesser SR and the test formulation are presented in table 1 while the values of the pharmacokinetic parameters, k_e, t½ and Vd/f of the two

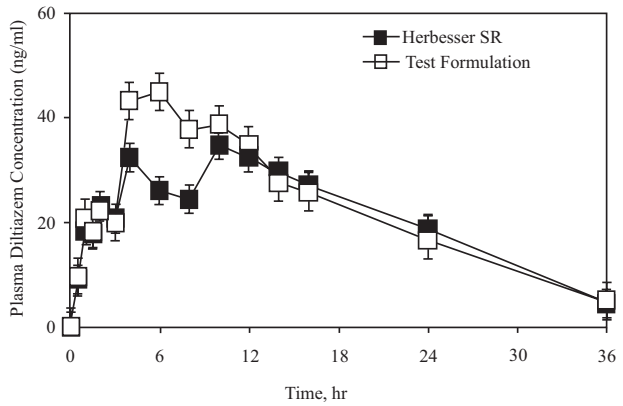

Fig. 2: Mean plasma diltiazem concentration versus time profiles of Herbesser SR and test formulation. Mean ± SEM (vertical bar), N=6.

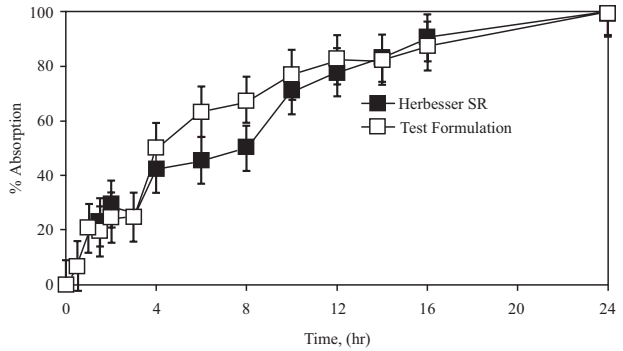

Fig.3: Mean *in vivo* diltiazem absorption versus time profiles of Herbesser SR and test formulation. Mean ± SEM (vertical bar), N=6.

formulations are given in the table 2. The values were similar and were not significantly different. Moreover, the pharmacokinetic parameters values obtained were in good agreement with those reported in the literature (Christrup *et al.*, 1992; Murata *et al.*, 1989). The parameters T_{max} and $AUC_{0-\infty}$ are related to the respective rate and extent of drug absorption, while C_{max} is related to both processes (Grahnen, 1984). The mean T_{max} values for the Herbesser SR (10.7 ± 4.8 hours) was higher compared to the test formulation (7.7 ± 2.9 hours) indicating a relatively slower absorption rate of Herbesser SR. Non parametric analysis using the Wilcoxon test showed a significant difference between the T_{max} values of Herbesser SR and the test formulation ($p<0.05$). T_{max} in the present situation may not be reliable estimate of rate of diltiazem absorption due to multiple peaks observed in the plasma concentration.

Relatively wide inter-subject variation was observed in the values of the parameters $AUC_{0-t,}$ $AUC_{0-\infty}$ and C_{max}, which could be attributed to differences in body weight and drug disposition among the subjects. No statistically difference was observed between the log transformed $AUC_{0-\infty}$ values as well as log transformed C_{max} values ($p > 0.05$) of the two preparations. In addition, the 90% confidence interval (CI) for the ratio of the log transformed $AUC_{0-\infty}$ values of the test formulation over those of Herbesser SR was estimated to be between 0.90-1.24, being within the acceptable bio-equivalence interval of 0.80 and 1.25 (USP 24, 2000). The mean C_{max} value of the test formulation (51.7 ± 14.9 ng/ml) was higher than Herbesser SR (39.7 ± 10.7 ng/ml) and the 90% CI of C_{max} estimated in the present study was in the range of 0.90-1.87. Again analysis using C_{max} may be an unreliable due to the presence of multiple peaks in both the formulations (Steinijans and Hauschke, 1992).

For bioequivalence analysis, all the available parameters should be considered (Khoo *et al.*, 1985). Numerous reports have emphasized the benefit of using C_{max} /AUC and some even proposed that the absorption rate metrics should be modified to replace C_{max} and T_{max} with the C_{max} /AUC ratio. In contrast, the indirect metrics of C_{max} and T_{max} were found to be insensitive to assess the rate of absorption in bioequivalence studies (Rostami-Hadjegan *et al.*, 1994). In this study, C_{max} /AUC analysis gave a 90% CI of 1.07-1.39 which was also not within the acceptable range of 0.80 and 1.25 for bioequivalence. However, the examined parameter seemed to be better than C_{max} for the assessment of rate of absorption of SR products.

The mean *in-vivo* diltiazem absorption versus time profiles of the formulations is presented in fig.3. Both the formulations showed fluctuations at certain points but a slower absorption rate from the Herbesser SR capsules could be observed. It is interesting to note that in-vitro release rate of two formulations were closely similar but the in-vivo absorption of the Herbesser SR was comparatively slower than the test formulation from 4-12 hours. Diltiazem has high first pass effect and is thus metabolized by liver or the intestinal mucosal cells prior to reaching general circulation. It might be possible that the Herbesser SR faced more extensive first pass metabolism due to the slower rate of absorption. On the other hand, the higher rate of drug released from the test formulation may saturate the metabolic pathways and hence more drug was capable of reaching the systemic circulation. This was reflected by the ratio of the $AUC_{0-\infty}$ values of the test preparation over those by the reference preparation which has a mean value of about 1.07.

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COLORIMETRIC DETERMINATION OF INDOLIC DRUGS

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A colorimetric method has been developed for the quantitative determination of the rescinnamine, reserpine upto (-10^{-4}M), Yohimbine on complexation with bromothymol blue. The coloured complexes exhibit absorption maxima in the region 415-416 nm. The RSD (Relative Standard Deviation) of the method is 2.02%. The method is simple, easy, rapid and convenient for routine analysis of the indolic drugs.

Keywords: Colorimetric determination, indolic drugs, coloured complexes.

INTRODUCTION

Indolic drugs are highly complex naturally occurring nitrogenous compounds, possessing an indole nucleus. Many of these are alkaloids and are used as antihypertensive and tranquilizing agents (Raffauf & Flagler, 1960 and Rosh *et al*., 1990). Some important examples are rescinnamine, reserpine and yohimbine. The indole alkaloids have been the subject of considerable study as described in a comprehensive monograph (Rahman & Basha, 1983). Indole nucleus have been shown to act as a good electron donor in charge transfer complexes (Foster & Hanson, 1964; Fieser & Fieser, 1973; and Kutney & Redcliff, 1975). Intense colors are usually associated with charge transfer complexes in the solid as well as in solution (Mulliken, 1939; Berg & Lam, 1964; Hammond & Burkardf, 1970; Gyorgyi, 1960 and Hutzinger, 1969). This property has been used for the quantitative determination of indolic drugs (Manzar and Alam, 1992). Spectrophotometric method for the determination of indole and its derivatives as charge transfer complexes have been reported (Manzar and Kost, 1980, Hager *et al*., 1986 and Borazan & Ajeena, 1988). The objective of this study is to develop a spectrophotometric method for the indolic drugs based on the principle of charge transfer complexes with the acceptor bromotyhymol blue.

RESULTS AND DISCUSSION

Indole nucleus acts as a good electron donor in charge transfer complexes. A considerable amount of work involving charge transfer properties of indoles is available because several biologically important compounds possess the indole ring system follow charge transfer phenomenon