Biopharmaceutical Evaluation of Oral Controlled Release Verapamil Hydrochloride Microcapsules

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Biopharmaceutical behaviour of two controlled release microcapsule formulations of verapamil hydrochloride were described as evaluated in rabbit in comparison with drug material. Microcapsule formulation c coated with ethylcellulose and formulation j coated with Eudragit RS were also compared using pharmacokinetic parameters. Upon t test a significant difference between the formulations was observed in the parameters, K, T 1/2, AUC (o-α) MRT and bioavailability (F) but not in Vd, Cmax, Tmax and in all cases a highly significant difference was noted with drug material except in Vd. Wagner-Nelson method was employed for the assessment of *in vivo* sustained release absorption profiles of drug from the formulations. From the method of residuals, absorption lag time, absorption half life and absorption rate constant were estimated.

The aim of any controlled release system is to reduce the Cmax value to decrease side effects and to increase duration of steady-state plasma concentration[1-4]. The present study was taken up to evaluate the biopharmaceutical behaviour of two controlled release microcapsule formulations of verapamil hydrochloride based on *in vitro* dissolution studies described previously[5,6].

Healthy rabbits were utilised for the biopharmaceutical study and the pharmacokinetic parameters obtained were compared between the experimental products and the pure drug. The *in vivo* absorption profiles of the formulations calculated using the Wagner-Nelson method[7] were further compared.

Male albino rabbits of 1.6 to 2.0 Kg were selected for the study. Drinking water and a commercial animal feed was made available throughout the study. All animals were fasted for 12 h prior to drug administration but water was allowed *ad libitum*. On the next morning verapamil HCl 50 mg/kg body weight was administered orally to each group of the three rabbits. Food was allowed 4 h after drug administration. For determination of verapamil hydrochloride, 2 ml blood samples were drawn immediately before administration and 0.5, 1.0, 2.0, 4.0 and 8.0 h after administration.

Following the same technique, verapamil microcapsule formulation c (composed of ethylcellulose, drug content. 23.1% of same dose (50 mg/kg) was administered to each rabbit of the second group. Blood samples were drawn from each rabbit at 2.0, 4.0, 8.0, 12.0 and 24.0 h after administration. The process was repeated for verapamil microcapsule formulation j (composed of Eudragit RS, drug content - 18.2%) to each rabbit of third group and blood samples were collected at same time intervals.

Verapamil hydrochloride, 30 mg was dissolved in 5 ml of water for injection and a volume equivalent to 4 mg/kg body weight (1.33 ml) was injected slowly at a rate of 0.5 ml/min, through marginal ear vein to one rabbit. Blood samples were withdrawn at 0.1, 0.25, 0.5, 1.0, 2.0, 3.5, 5.0 and 7.0 h after injection. The blood samples were collected in citrated tubes and rapidly centrifuged. The plasma fraction was separated and frozen immediately till the analysis[8] was performed.

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The pharmacokinetic parameters such as, maximum concentration of drug in plasma, Cmax the time for the drug to reach maximum concentration in the plasma after drug administration, Tmax, were computed directly from the measured plasma concentration data. The elimination rate constant (K) was estimated from the terminal slope of the individual plasma concentration - time curve after logarithmic transformation of plasma concentration values. Elimination half- life, T1/2, was calculated from the quotient 0.693/K. Areas under the plasma drug concentration-time curve AUC(0-α) was calculated using the trapezoidal rule and the latter by dividing the last measurable plasma drug concentration with elimination rate constant[9]. The apparent volume of distribution (Vd), was calculated as:

Vd = Dose/AUC.K. The Wagner-Nelson method was employed to calculate the *in vivo* absorption profiles of pure drug and two formulations. To assess the degree of retardation of drug release from different formulations, mean residence times (MRT) were calculated[10,11]. The graphic method of residuals was employed to test whether or not absorption is a first-order process and, if so, to determine the absorption half-life, T1/2a. The absorption half-life was calculated as the time for the residual value to diminish by one-half. The corresponding absorption rate constant, Ka was calculated from the slope of the plot of the residuals against time. From the same method of residuals, absorption lag time (Tlag) was also determined. Assuming the clearance remained constant the oral bioavailability (F) of the formulations were determined from AUC (o-α) (i.v) value of pure drug. Statistical evaluation were carried out using the paired t-test. The difference between two means was considered statistically significant when p(0-α)0.05.

The mean pharmacokinetic parameters after oral administration of pure verapamil hydrochloride and its formulations c (V:Eth = 1:4) and j (V:RS = 1:5) are given in Table 1. Data are reported in all cases as mean± stand-

TABLE 1 : PHARMACOKINETIC PARAMETERS OF ORAL VERAPAMIL ADMINISTRATION

Parameters	Pure Drug	Form(C)	Form (j)
K (1/h)	0.269±0.050	0.084±0.007	0.116±0.014
	(18.6%)	(8.3%)	(12.0%)
T ($^1/_2$h)	2.63±0.45	8.25±0.64	6.03±0.62
	(17.3%)	(7.8%)	(10.3%)
A U C.(o-α)	36.39±0.80	78.77±2.01	71.39±3.00
mcg.h/ml	(2.2%)	(2.5%)	(4.2%)
Vd (L/Kg)	5.23±0.98	7.56±0.71	6.122±0.886
	(18.9%)	(9.3%)	(14.4%)
Cmax	7.087±0.750	4.204±0.550	5.285±0.750
(mcg/ml)	(10.6%)	(13.1%)	(14.2%)
Tmax (h)	0.83±0.29	5.30±2.30	3.30±1.10
	(34.6%)	(43.3%)	(35.0%)
M R T (h)	4.23±0.57	14.42±1.21	9.95±0.65
	(13.5%)	(8.4%)	(6.6%)
F(%)	38.3±0.8	83.0±2.1	75.1±3.2
	(2.2%)	(2.6%)	(4.3%)

Pure drug and formulations were administered orally (50 mg/kg) in healthy rabbits and various pharmacokinetic parameters were produced as mean± standard deviation (n=3). Value in the parenthesis represents coefficient of variance.

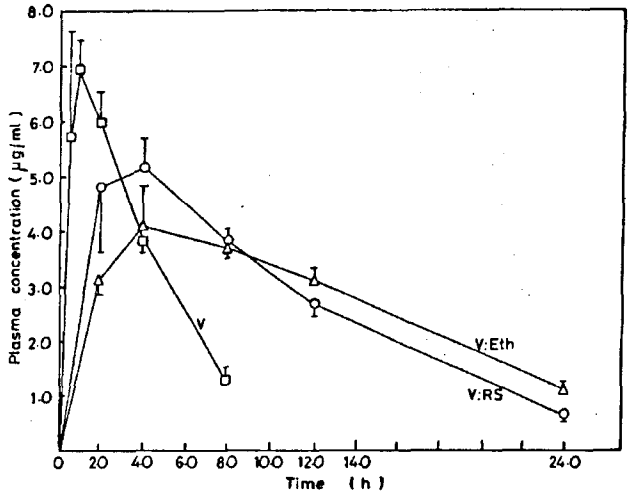

Fig. 1 : Mean plasma concentration-time profiles of verapamil HCl after oral administration of 50 mg/kg in healthy rabbits of verapamil (V), formulation (c) V:Eth = 1:4, formulation (j) V:Rs = 1:5. Mean± Standard deviation (n=3).

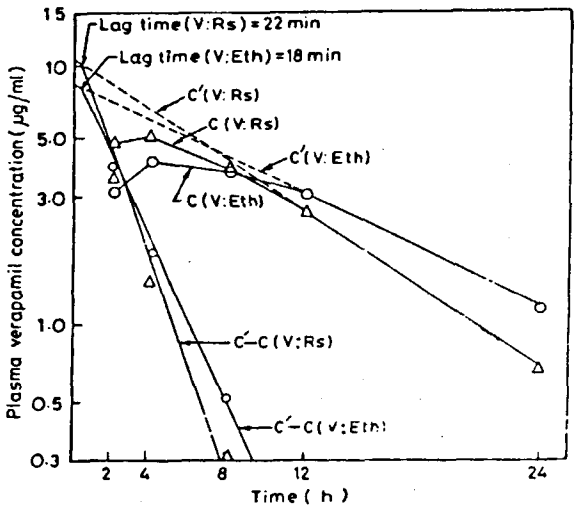

Fig. 2 : Estimation of absorption half life (T1/2a) and lag time by method of residuals, following oral administration of verapamil HCl, (50 mg/kg) :
formulation (c) (V:Eth = (1:4) - T1/2a = 2.44 h
formulation (j) (V:RS = (1:5) - T1/2a = 1.91 h

ard deviation with per cent coefficient of variance. The plasma profiles of verapamil hydrochloride and formulations c and j are shown in fig 1., Fig. 2 depicts the estimation of absorption half life (T1/2a) and lag time (Tlag) by the method of residuals. All profiles of formulations are reflective of a slow and sustained rate of drug absorption with reference to pure drug and plasma drug concentration was still detectable at 24 h. The retardation of dissolution rate of formulation c compared with formulation j of verapamil microcapsule resulted in a corresponding retardation of the elimination rate (opposite in case of T1/2) and extent of absorption and consequently in an enhanced AUC. The AUC (0-α) of verapamil formulation c was significantly different from that of formulation j ($p<0.05$). The numerical values of parameters K and T1/2 Table 1 of the two formulations were observed to vary significantly with each other and with the standard drug material ($p<0.05$). The Cmax and Tmax values of formulations slightly changed with change of AUC. Cmax slightly increased with decrease of AUC in the formulations. No statistically significant differences could be detected among the formulations. But Cmax of pure drug showed significantly higher values then those of formulation c ($p<0.05$) and formulation j ($p<0.05$). The Tmax value increased in formulation c ($p<0.05$) and formulation j ($p<0.05$) than that of reference drug. Significantly increased MRT values were noticed for controlled release variants in comparison to the standard drug. Statistically significant differences could also be found among the formulations. MRT values increased with the increasing AUC values. Formulation c with longer MRT showed more sustained or prolonged absorption of the drug with a constant first order rate of elimination. AUC is directly related with bioavailability and as per expectation bioavailability of the formulations increased in the same order as the AUC. Bioavailability of the formulations significantly increased in comparison with the pure drug ($p<0.05$). Statistically significant differences were observed in the inter-formulation variation also ($p<0.05$). Apparent volume of distribution slightly increased with the bioavailability, no statistically significant differences were detected between the formulations. The differences between the Tlag values of the formulations was 4 min which is the indication of approximation of the beginning of absorption fig. 2. But they delayed 12 to 16 min in the start of absorption from the pure drug itself. The value of T1/2a of formulation c is greater than formulation j and reversely in Ka as found from the above figure. Therefore, the rate limiting step is the release of drug from the present formulation variants rather than the absorption. The use of a single unit to deliver a drug at a controlled rate cannot lead to a treatment by reducing dosing frequency without loss of bioavailability unless the drug has a long biological half-life[12].

In conclusion, the assessment of biopharmaceutical evaluation was successfully applied to the microcapsule formulations of verapamil hydrochloride. The rate of absorption appeared to be more sustained, resulting in a relatively more uniform plasma concentration profile of the drug about at least 12 h. About twice and more bioavailabilities were noted with sustained release formulations even though the drug has substantial first pass metabolism. The results indicate that it is possible to make a once a day oral controlled release dosage form for verapamil hydrochloride.

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Chemical Components of *Melia azedarach* Stems

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***Melia azedarach* stems have been shown to contain melianin B, sendanolactone, ohchinin acetate, surianol and 3α-hydroxy-4,4,14 α -trimethyl-5α-pregn-8-en-20-one. The first three have already been reported from this plant. The last two compounds have already been reported from *Suriana maritama* and *Fomes officinalis* respectively.**

Melia azedarach, known as bakain, drek and mahanimb, belongs to the family Meliaceae. It resembles neem in many respects and possesses antimicrobial, insecticidal and nematicidal properties. An aqueous extract of its leaves increases haemoglobin[1]. Its stems have been reported to contain melianin A, melianin B, nimbolin A and nimbolin B[2,3]. We have undertaken a reinvestigation of its stems.

The dry stems of *Melia azedarach* (12 kg) were procured from the Landscape, CCSHAU, Hisar and extracted with hot methanol. The methanolic extract (300 g) was subjected to silica gel (60-120 mesh) column chromatography. Elutions with petroleum ether, benzene, ethyl acetate, methanol and their mixtures in the order of their

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