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GASTROINTESTINAL ABSORPTION OF DRUGS (CARBAMAZEPINE –CBZ): *IN Vivo - In Vitro* CORRELATIONS USING A COMMERCIAL ABSORPTION SIMULATOR

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ABSTRACT

The rate of intestinal absorption of carbamazepine (CBZ) was studied using the Sartorius Absorption Simulator. Difiusion rate constants (k_d) of 7.43 (±0.32) x 10^{-3} cm.min^{-1} and 7.44 (±0.2) x 10^{-3}cm.min^{-1} were obtained using Sörensen phosphate buffer pH 7.5 and the same buffer containing 0.4% BSA respectively as simulated plasma. These k_d values were converted into absorption rate constants (K_i) of 7.43 x 10^{-2}min^{-1} and 7.44 x 10^{-2} min^{-1} (for humans, G-factor 10) and 3.34 x 10^{-2}min^{-1} and 3.35 x 10^{-2}min^{-1} (for rats, G-factor 4.5). These results show that the presence of 0.4% BSA in the artificial plasma produces no apparent difference in the diffusion process. A correlation was also made between the simulated absorption rate constant (K_i) from this *in vitro* method and that obtained from an *in situ* rat gut technique, 5.29±0.8min^{-1} or 3.23±0.79min^{-1}g^{-1} (as per dry weight of the gut). These results thus suggest that the measurement of diffusion rates through artificial membranes could be a useful predictive tool for the assessment of the availability in man of new lipophilic compounds.

KEYWORDS: Intestinal absorption, sartorius absorption simulator, carbamazepine.

INTRODUCTION

Gastrointestinal absorption has been defined as the net movement of a substance from the gastrointestinal lumen into the systemic circulation (Houston and Wood, 1980). The systemic bioavailability of a drug administered by routes such as oral, sublingual and parenteral depends on efficient absorption from the sites of administration. Several methods used to determine the rates and extent of gastrointestinal absorption of drugs are available. The importance of *in vitro* techniques for these measurements has been established (Bridges et al., 1976; Koster and Noordhoek, 1983; Bermejo et al., 2004). Stricker (1971a) developed artificial lipid membranes which were claimed to simulate the gastrointestinal barrier when the absorption is by simple passive diffusion. This technique forms the basis of a commercial absorption simulator for which good correlations between drug absorption *in vitro* and *in vivo* have been claimed (Stricker, 1971b; 1973).

Most of the drugs so far studied with the absorption simulator could be classified as hydrophilic compounds (Stricker, 1971c; Braybrooks et al., 1974; Jones & Bye 1979; Mura et al., 1986). For this class of drugs, only one experiment involving the measurement of the passage of the drug from the artificial intestinal juice through the artificial lipid barrier to the artificial plasma is required. Based on the good correlations reported for these hydrophilic compounds, and since no correlations have so far been made for lipophilic compounds (which dissolve in the membrane), the present investigation was therefore to correlate the kinetics of intestinal drug absorption *in-vivo – in vitro* using the absorption simulator for lipophilic compounds. For this investigation, carbamazepine (CBZ), a widely used drug for prevention of seizures and for trigeminal neuralgia, was chosen as a model lipophilic drug (Kutt & Paris-Kutt, 1982). In the preliminary work, carbamazepine was found to concentrate in the lipid phase of the barrier (membrane) to about 50%.

For the absorption simulator to be adapted for lipophilic compounds, Stricker (1971b) suggested that the experimental technique used for hydrophilic compounds should be modified. Thus, for the present work, the technique used required two separate tests, each measuring the partitioning or binding of the drug from either the artificial intestinal fluid (phosphate buffer, pH 6.0) or artificial plasma (phosphate buffer pH 7.5) to the membrane.

MATERIALS

Carbamazepine was obtained from Novartis Pharmaceutical, Batch No. PHO 4560/A. The Sartorius absorption simulator was used with the membrane-forming chemicals [mainly a mixture of caprylic acid (80 parts) and lauryl alcohol (20 parts)] and materials as supplied by the manufacturers (Sartorius Membrane filter GmbH Germany). All drug assay was carried out on a Perkin Elmer 552 spectrophotometer at λ_{max} 285nm. Bovine serum albumin (BSA) fraction V was used for experiments requiring protein.

METHOD

Determination of the *in vitro* diffusion and absorption rate constants using the Sartorius Absorption Simulator

For this determination, two sets of experiments were carried out. The experimental procedure required the measurement of the accumulation of the drug to the membrane from both the artificial intestinal juice (Sörensen phosphate buffer, pH 6.0) and artificial plasma (Sörensen phosphate buffer, pH 7.5).

For the first experiment, a solution of CBZ (51.2 µg/ml) in the artificial intestinal juice (100ml) and diffusion cell fitted with a membrane having an area of 40cm^2 were used. The same amount of drug in 100ml of artificial plasma was used for the second set. Samples (2ml) were taken from each compartment at 30 minutes intervals until concentration equilibrium between the aqueous and lipid phases was attained and assayed by UV. The compartment temperature was maintained at 39±1°C through out the duration of the experiment. Another set of experiments was repeated analogously but using artificial plasma containing 0.4% w/v bovine serum albumin (BSA). For this set, the samples were extracted into dichloromethane at pH 6.8, washed with both alkali and acid and then assayed in 96% ethanol

The diffusion rate constant (k_d) was calculated from the formula (Stricker, 1973)

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$$k_d \quad k_{do}^{*} = \frac{\beta' \cdot \beta'' \cdot B'' (1 - B') \times 2.3 \times V_{lo}}{\beta' B' + \beta'' B} \quad (cm\ min^{-1}) \qquad (1)$$

F

where V_{lo} = Initial volume (100ml)

F = Effective barrier area ($80cm^2$)

$B' = \frac{C_{leq}}{C_{lo}}$; $B'' = \frac{C_{lleq}}{C_{llo}}$

(experimentally determined equilibrium concentrations in the particular aqueous phase)

β' and β'' are the respective slopes from the semilog. plot of the ratio of concentration versus time for the two phases (equation 2)

$$\log C_x/C_{xo} - B_x = \beta_x t + \log (1 - B_x) \qquad \ldots\ldots\ldots\ldots (2)$$

(x denotes either of the phases; I or II)

* The experimental procedure for lipophilic substances makes k_{do} very small and therefore not necessary Thus (k_d-k_{do}) approximates to k_d

As stated by Stricker, the absorption rate constant (K_i) is directly proportional to the diffusion rate constant (k_d) and the proportionality constant is the G- factor This factor is an empirical factor which depends on the site of absorption. Stricker proposed the following values as G-factor (10.0 for human small intestine and 4.5 for rat small intestine). Thus:

$$K_i \alpha k_d \ ; \qquad K_i = G k_d \qquad \ldots\ldots\ldots\ldots (3)$$

Determination of absorption rate constant by *in situ* rat gut technique

The procedure of Doluisio et al. (1969) was used on male Wister albino rats weighing 230 – 250g, fasted overnight but with access to water After washing the gut with the perfusion solution, 11ml of the drug solution (105 µg/ml CBZ in phosphate buffer pH 6.0) was introduced into the gut through the duodenal end and pumped through into the ileal syringe. A sample (1ml) was taken out at time zero minutes and the rest of the solution returned to the gut. At 10minutes intervals (for up to 1hr) the solution in the intestine was pumped into either syringe and a 0.5ml sample removed for assay

RESULTS AND DISCUSSION

Carbamazepine (5-carbamyl-5H-dibenzo[b,f]azepine, CBZ) is a neutral lipophilic compound Table 1 shows the experimentally determined concentrations of CBZ and the ratio of these concentrations at different time intervals in the different compartments (C_l, C_{ll} and C_{ll}). The term Ceq was obtained when concentration equilibrium was attained, i.e., when there was no further change in the concentration of CBZ in the respective aqueous phase (after about four hours in each case). These data were used to obtain figures 1A to 3A which show the rate of accumulation of CBZ in the membrane using the absorption simulator Figures 1B to 3B are the semi-log plots of the ratios of concentration versus time according to equation 2 above The slopes (β_x) determined from these straight line graphs were used in calculating the diffusion rate constants (k_d) using equation 1 These diffusion rate constants (1), the corresponding absorption rate constants (2) calculated for both humans and rats and absorption rate constants obtained from the *in situ* rat gut technique (3) are shown in Table 2

In designing the *in vitro* experiments, besides using pure phosphate buffer (pH 7.5) as the artificial plasma, an attempt to elucidate the possible role of the plasma proteins was made by carrying out similar experiments using a 0.4% solution of BSA in the phosphate buffer as the artificial plasma This was found to give the same diffusion rate constant (7.43×10^{-3} $cm.min^{-1}$ and 7.44×10^{-3} $cm\ min^{-1}$) respectively. Although albumin constitutes about 4% of plasma proteins, the use of a similar concentration of BSA proved impracticable for the present work because of excessive frothing of the solution. Nevertheless, this result could infer that the presence of the 0.4% BSA in the artificial plasma had no apparent effect on the diffusion process. This is in support of earlier reports that though CBZ is 75% bound to plasma proteins, the relatively low association constant of the complex made the binding to be of no practical importance (Bertilsson, 1986)

For the conversion of diffusion rate constants into absorption rate constants, the empirical conversion factor, G-factor of 10.0 (for human GI tract) and 4.5 (for rats) were used (Stricker, 1971b). Using these conversion factors, values of 7.426×10^{-2} min^{-1} and 7.439×10^{-2} min^{-1} (for humans) and 3.342×10^{-2} min^{-1} and 3.348×10^{-2} min^{-1} (for rats) were obtained. In their study on CBZ using human subjects, Kahela *et al* (1983) obtained absorption rate constants of $(2.37 - 0.87) \times 10^{-2}$ min^{-1} for anhydrous compound and 2.9×10^{-2} min^{-1} for the dihydrate (using a dose of 200mg with 100ml H_2O). Levy *et al.* (1975) determined a value of $3.98 \times 10^{-2} min^{-1}$ (for solution) and 6.72×10^{-3} (for tablet). A comparison of these values (especially the Levy *et al.* result for solution) with those obtained from this *in vitro* method using G-factor of 10 for humans, shows that the present results are twice as large. A similar comparison between the normalised absorption rates $3.23 (\pm 0.79) \times 10^{-2} min^{-1} g^{-1}$ calculated from the *in situ* rat gut technique (in per unit weight of dry gut) and the *in vitro* results (Table 2) gives good correlation.

Generally, the differences observed in the above results could be accounted for by the lipid nature of the artificial barrier and the empirical constant (G) employed to convert the experimental diffusion rates (k_d) into simulated absorption rates (K_i) (Stricker, 1973). In the *in vitro* method, the amount of drug bound to the lipoidal membrane was taken into consideration in calculating k_d and K_i. The *in situ* method measures accurately the disappearance of drug from the gut segment but does not reveal how much has been stored in the absorbing membranes The rate of this accumulation, if not linear, could introduce errors in the numerical value of K_i. Similar argument applies to the data obtained using human subjects. Nevertheless, the fact that a relatively good comparison was obtained in this present study suggests that as has been found for hydrophilic drugs, measurement of diffusion rates through artificial lipid membranes could be a useful predictive tool for the assessment of the availability in man of new lipophilic compounds

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Table 1: Experimentally determined concentration of CBZ in the different compartments

	AIF		AP		AP_{BSA}	
Time (min)	C_I (μgml^{-1})	$\frac{C_I}{C_{Io}} - B_1$	C_{II} (μgml^{-1})	$\frac{C_{II}}{C_{IIo}} - B_2$	C_{II}' (μgml^{-1})	$\frac{C_{II}'}{C_{IIo}} - B_2'$
0	51.21	0.727	51.21	0.660	44.77	0.651
30	33.05	0.373	37.14	0.385	32.87	0.385
60	24.27	0.201	30.01	0.246	26.70	0.246
90	19.82	0.114	26.89	0.185	23.57	0.176
120	17.87	0.076	23.71	0.123	21.56	0.132
C_{eq}	**13.96**		**17.42**		**15.67**	
Slope = $\beta \times 10^{-3}$	8.254		5.925		5.753	
Intercept	0.6762		0.6111		0.5976	

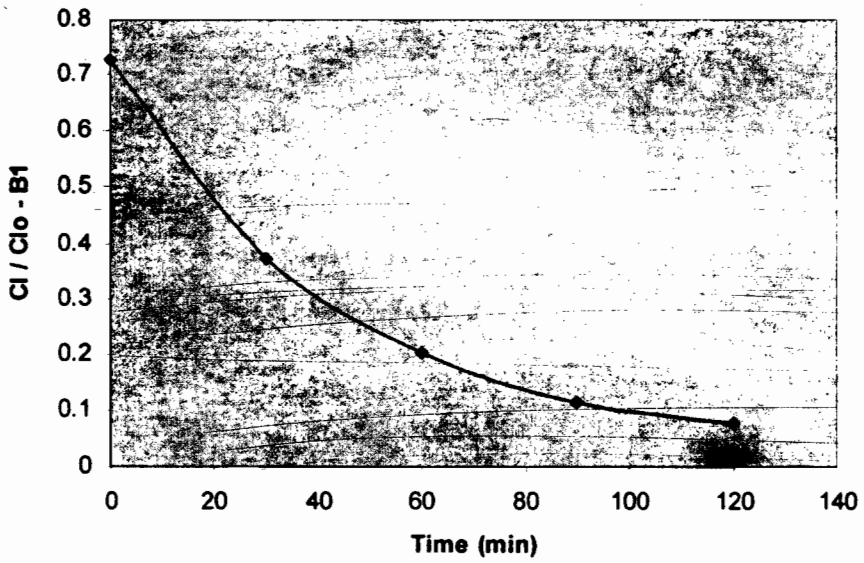
Notes:
AIF = Artificial intestinal fluid (phosphate buffer pH 6.0); **B_1 (C_{eq}/C_{Io} = 0.273)**
AP = Artificial plasma (phosphate buffer pH 7.5); **B_2 (C_{eq}/C_{IIo} = 0.340)**
AP_{BSA} = Artificial plasma (phosphate buffer pH 7.5 plus 0.4% w/v BSA);
B_2' (C_{eq}/C_{IIo}' = 0.350)

Table 2: Diffusion (1) and absorption (2) rate constants of CBZ using Sartorius absorption simulator and absorption rate constant from *in situ* rat gut technique (3)

		(1)	(2)	3(c)	
Artificial Intestinal Fluid	Artificial Plasma	kd x 10^{-3} cm min^{-1} ± SD	ki x 10^{-2}cm min^{-1} ± SD	ki x 10^{-2} min^{-1} ± SD	ki x 10^{-2} min^{-1} g^{-1}** ± SD
Phosphate buffer pH 6.0 + CBZ	Phosphate buffer pH 7.5 + CBZ	7.426 ± 0.32	7.426 ± 0.32[a] 3.342 ± 0.32[b]	5.29 ± 0.8	3.23 ± 0.79
	0.4% BSA in phosphate buffer pH 7.5 + CBZ	7.439 ± 0.2	7.439 ± 0.2[a] 3.348 ± 0.2[b]		

Notes
(a) Calculated absorption rate constants for humans based on a G-factor of 10
(b) Calculated absorption rate constants for rats based on a G-factor of 4.5
(c) Mean of 6 rats. ** K_i from *in situ* rat gut technique after normalisation for weight of dry gut.

(A)



(B)

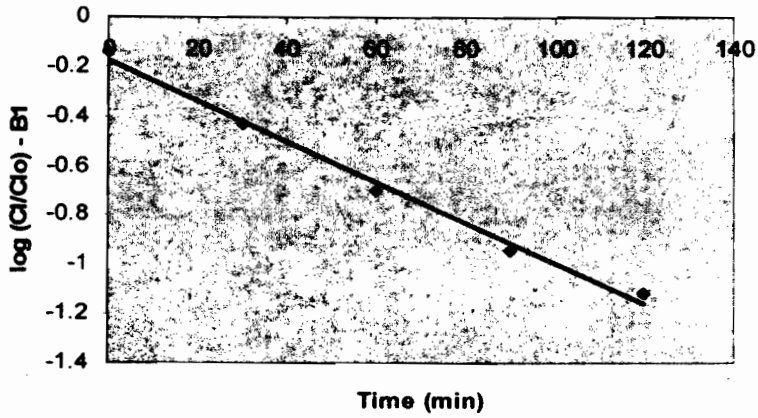
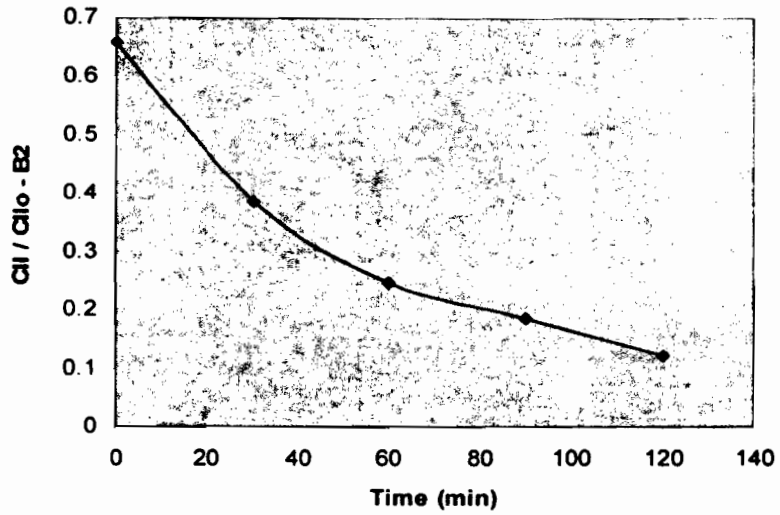


Fig. 1: Plot of concentration of CBZ (A) and log of concentration of CBZ (B) in artificial intestinal fluid (phosphate buffer pH 6.0) versus time

(A)



(B)

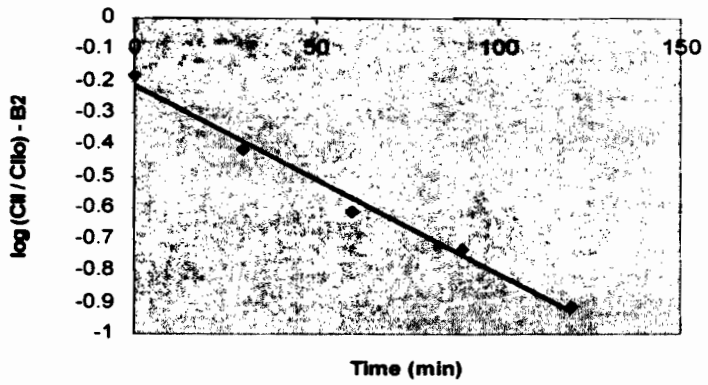
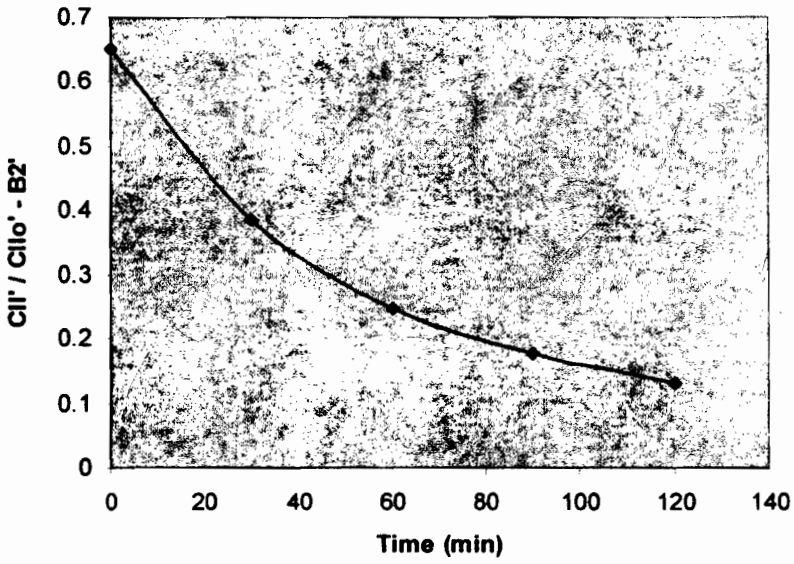


Fig. 2: Plot of concentration of CBZ (A) and log of concentration of CBZ (B) in artificial plasma (phosphate buffer pH 7.5) versus time

(A)



(B)

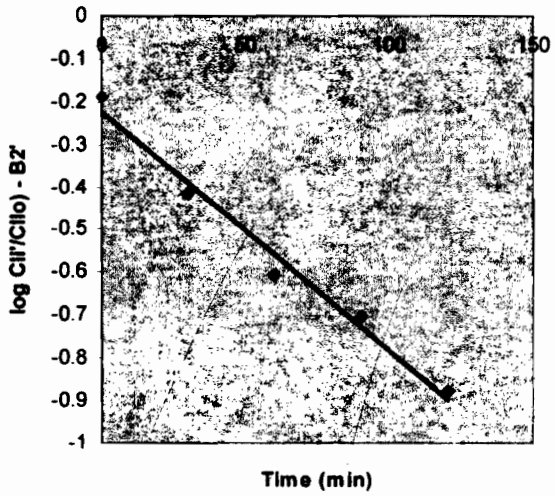


Fig. 3: Plot of concentration of CBZ (A) and log of concentration of CBZ (B) in artificial plasma (phosphate buffer pH 7.5 in the presence of 0.4% BSA) versus time

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