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Correlation of bioavailability in man with simulated absorption data for three doxantrazole preparations

H. JONES* AND A. BYE†

* *Pharmaceutical Development Laboratories, The Wellcome Foundation Ltd., Temple Hill, Dartford, Kent, DA1 5AH, U.K.* † *Department of Clinical Pharmacology, The Wellcome Research Laboratories, Beckenham, Kent, BR3 3BS, U.K.*

The in vitro and in vivo availability of doxantrazole, a potential antiallergic compound has been evaluated. A solution was significantly less bioavailable than either tablet or suspension formulations and it is suggested that this is associated with the large volume of the solution vehicle altering the hydrophilicity of the gastrointestinal fluids. In vitro availability was determined from absorption rate constants and absorption profiles obtained using the Sartorius absorption and solubility simulators. A statistically significant correlation was found between the percentage absorbed in vitro at 1h and both total urinary recovery and area under plasma curve values in vivo. It is considered that in vitro determination of diffusion through artificial lipid membranes may be a useful predictive method of in vivo availability.

The importance of in vitro techniques to assess the availability in man of drug substances from oral pharmaceutical preparations is well established (Swarbrick 1970; Wagner 1971; Leeson & Carstensen 1974). Where correlations have been obtained with in vivo studies, the in vitro methods used have generally been those of dissolution and particle size measurement. However, simple dissolution procedures do not necessarily reflect the often complex relationship that exists in vivo between solution of the drug from a dosage form and the movement of the drug across the biological membrane of the gastrointestinal tract. Dibbern (1969) and Stricker (1971) developed artificial lipid membranes which were claimed to simulate the gastrointestinal barrier when the absorption process was by simple passive diffusion and using such membranes Stricker (1971) obtained good agreement between in vitro and in vivo absorption rates for a variety of neutral, acidic and basic compounds.

In this work the bioavailability of three formulations (tablet, suspension and solution) each containing 200 mg doxantrazole (3-(5-tetrazolyl)-thioxanthone-10,10-dioxide), a potential orally active antiallergic compound (Batchelor et al 1975; Haydu et al 1975) has been measured and correlated with in vitro data using the Sartorius absorption and solubility simulators (according to Stricker).

MATERIALS AND METHODS

Materials

The tablets contained 200 mg of doxantrazole in a total weight of 580 mg: the excipients being dicalcium phosphate (U.S.N.F.), starch (B.P.), povidone (B.P.C.) and magnesium stearate (B.P.).

The suspension consisted of a sorbitol solution (B.P.C.) containing 200 mg doxantrazole in 10 ml with carbomer (B.P.C.) as the suspending agent. The solution contained 200 mg of doxantrazole in 100 ml of a mixture of propylene glycol (B.P.), alcohol (B.P.) and sorbitol solution (B.P.C.).

METHODS

Determination of in vitro absorption rate constants

One tablet, 10 ml of suspension or 100 ml of solution was mixed with 100 ml of a pH 1·2 solution (0·6% v/v conc. hydrochloric acid in deionized water) or 100 ml of a pH 6·0 buffer solution (0·15% w/v disodium orthophosphate, 0·80% w/v potassium dihydrogen-orthophosphate in deionized water). The resultant mixture was maintained at 37 °C for 60 min with shaking to effect partial (pH 1·2) or complete (pH 6·0) solution of the drug, then 100 ml (Phase I) transferred to a Sartorius absorption simulator (V. A. Howe & Co. Ltd.). The artificial gastric lipid barrier was used for experiments at pH 1·2 and the artificial intestinal lipid barrier for experiments at pH 6·0. In both cases the simulated plasma solution (Phase II) consisted of 100 ml of a pH 7·5 buffer solution (2·05% w/v disodium orthosphosphate, 0·28% w/v potassium dihydrogen-orthophosphate in deionized water). Diffusion cells of 40 cm^2 were used. Samples were removed from both sides of the lipid barriers at 0, 30, 60, 90 and 120 min, diluted with 0·1 M sodium hydroxide and assayed spectrofluorometrically (λ ex. 325 nm, λ em. 525 nm) against a standard solution of 1 mg $litre^{-1}$ doxantrazole in

* Correspondence.

0·1 M sodium hydroxide solution. The diffusion rate constants, K_d and the simulated absorption rate constants, K_I were calculated from the formulae (absorption simulator hand book):—

$$Kd = \frac{CII_2 - CII_1}{T_2 - T_1} \times \frac{1}{CI_0} \times \frac{100}{F} \text{ cm min}^{-1} \quad (1)$$

where T_2 and T_1 = time (min), CII_2–CII_1 = concn in Phase II at T_2 and T_1; CI_0 = concn in Phase I at T_0; F = barrier area (cm^2).

$$K_I = G(Kd\text{–}Kd_0) \text{ min}^{-1} \quad \ldots \quad (2)$$

where G = 4·3 for artificial gastric barrier, 10·0 for artificial intestinal barrier; $Kd_0 = 0{\cdot}7 \times 10^{-4}$ for artificial gastric barrier, $1{\cdot}8 \times 10^{-4}$ for artificial intestinal barrier.

Previous experiments with Phase I containing 10–100 mg $litre^{-1}$ of doxantrazole in the pH 6·0 buffer solution and using the artificial intestinal lipid barrier had shown K_I to be independent of initial starting concentration.

Determination of in vitro absorption profiles

Fig. 1 is a diagrammatic representation of the apparatus for the determinations. The dissolution chamber of the Sartorius solubility simulator (V. A. Howe & Co. Ltd.) was filled with 100 ml pH 1·2 solution plus 170 g plastic beads and equilibrated at 37 °C. Buffer solution (900 ml, pH 6·0) was pumped from the 1 litre flask at a rate of 1 ml min^{-1} through a 1 mm flow cell and back to the flask. The spectrophotometer, equipped with a program controller and chart recorder, was set to a zero absorbance value at 317 nm with the pH 6·0 buffer solution passing through the flow cell. Full scale deflection on the recorder was adjusted to 0·900 for the tablet and suspension experiments and 0·45 for the solution experiments, equivalent to 200 and 100 mg doxantrazole per litre respectively. One tablet was placed in the dissolution chamber and the experiment started by adjusting the sampling frequency to 0·55 min (calculated from the respective K_I value). The program controller recorded absorbance values at 10 min intervals. At 15 min the experiment was stopped and 1·2 g trisodium orthophosphate added to the chamber to raise the pH to 6·0. The sampling frequency was reset to 4·4 min and the experiment run for a further 165 min. Percentage absorbed values were read directly from the recorder. For the suspension 10 ml was added to 90 ml of pH 1·2 solution and the sampling frequencies set to 0·53 and 4·8 min respectively. For the solution, 100 ml was mixed with an equal volume of pH 1·2 solution and 100 ml of this mixture used. Sampling frequencies were 10·9 and 13·9 min respectively.

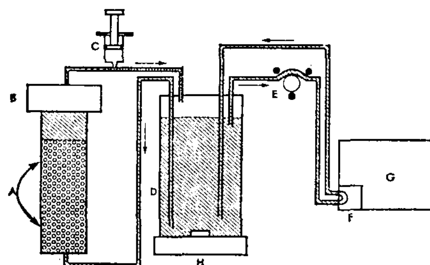

FIG. 1. Schematic diagram of the apparatus used for obtaining simulated absorption profiles. A = dissolution chamber, B = filtration unit, C = 5 ml syringe pump, of solubility simulator; D = 1 litre flask containing 900 ml pH 6·0 solution; E = peristaltic pump; F = 1 mm flow cell; G = spectrophotometer, programme controller and chart recorder; H = magnetic stirrer/hot plate.

Determination of in vivo pharmacokinetic parameters in healthy volunteers

Six healthy volunteers (3 male, 3 female) were given one of each of the formulations at intervals of one week. The administration of preparations was on a random crossover basis and each formulation was taken orally with 100 ml water by the volunteers in a standing position after a 12 h (overnight) fast. Blood and urine were sampled from 0 to 24 and 0 to 72 h respectively. Blood was placed into heparinized tubes and the plasma separated by centrifugation and stored at −20 °C until analysed. Urine was collected without preservative and a 20 ml aliquot stored at −20 °C until analysed. Drug content of both blood and urine samples was determined by gas liquid chromatography (Bye & Land 1975).

RESULTS AND DISCUSSION

The simulated absorption rate constants (K_I) for the three formulations are shown in Table 1. For both

Table 1. Simulated absorption rate constants (K_I)* for doxantrazole in three formulations, obtained from absorption simulator.

	K_I min^{-1}		
	Tablet	Suspension	Solution
Artificial gastric barrier, pH 1·2	0·085, 0·095	0·085, 0·105	0·0042, 0·0050
Artificial intestinal barrier, pH 6·0	0·010, 0·013	0·010, 0·011	0·0028, 0·0044

*Calculated for fasting state and gastrointestinal fluid volume, 100 ml.

tablet and suspension at each pH condition, the K_I values of the drug in the presence of excipients are similar. However, Table 1 shows that under the experimental conditions employed the K_I values for the drug from the solution was reduced about twentyfold at pH 1·2 and threefold at pH 6·0 when compared with the other two preparations. The differences in the K_I values between pH 1·2 and 6·0 for all three preparations can be accounted for by the differences in the lipid nature of the artificial gastric and intestinal barriers; the degree of ionization of doxantrazole at the two pH values (pK_a doxantrazole = 3·0) and the constants employed to convert the experimental diffusion rates (Kd) into the simulated absorption rates (2 above).

The simulated absorption profiles for the three formulations are presented in Table 2. After 3 h the solution, tablet and suspension absorption values were 52, 86 and 84% respectively. Although the K_I values were high at pH 1·2 the zero value for absorption can be explained by the very low aqueous solubility of doxantrazole at this pH (3 mg litre^{-1}). The comparatively short 15 min sampling at pH 1·2 was chosen to correspond to the anticipated in vivo conditions following administration of the three formulations with 100 ml water on a fasting stomach.

In vivo bioavailability data are summarized in Table 3. Areas under the plasma concentration curve (AUC) were determined by the trapezoidal method and the means calculated. Table 3 shows that the solution of doxantrazole was significantly less bioavailable than either the tablet or suspension preparations. This difference is reflected in the in vitro data presented in Tables 1 and 2 which suggests that an in vitro correlation is possible.

Table 4 shows the correlation coefficients obtained when peak plasma concentrations, AUC and urinary recovery with % absorbed in vitro at 1, 2, 3 h for the three formulations were compared. The in vivo, in vitro correlation between two of the bioavailability parameters (AUC and urinary recovery) and the

Table 2. Simulated absorption profiles (6 determinations) of doxantrazole for three formulations, obtained from solubility simulator.

Time (min)	pH	Tablet mean	Tablet s.e.m.	Suspension mean	Suspension s.e.m.	Solution mean	Solution s.e.m.
0	1·2	0	0	0	0	0	0
10	1·2	0	0	0	0	0	0
20	6·0	1	0·2	0	0	1	1·0
30	6·0	8	0·8	8	0	6	1·2
40	6·0	20	0·8	18	0	9	1·1
60	6·0	38	1·1	35	0·4	17	1·8
90	6·0	58	0·9	55	0·4	27	1·8
120	6·0	71	1·3	67	1·1	37	1·6
180	6·0	86	1·2	84	0·9	52	1·1

Table 3. Bioavailability data for six healthy volunteers following oral administration of 200 mg doxantrazole as tablet, suspension and solution.

	Tablet	Suspension	Solution
Peak plasma concn μg ml^{-1}			
Subject			
I	9·3	15·3	10·4
II	13·9	9·7	—
III	21·1	22·0	14·9
IV	19·1	19·1	10·4
V	19·3	8·4	15·2
VI	32·6	14·0	12·0
Mean	19·22	14·75	12·58
s.e.m.	3·21	2·15	1·05
AUC (plasma) μg ml^{-1} h (0–24 h)			
Subject			
I	40·0	28·0	31·5
II	65·3	76·4	—
III	80·4	65·0	50·3
IV	74·7	81·8	37·8
V	66·4	41·1	43·1
VI	72·4	63·4	42·2
Mean	66·53	59·28	40·98
s.e.m.	5·77	8·48	3·11
% Total urinary recovery (0–72 h)			
Subject			
I	23·0	23·0	17·2
II	26·2	28·1	22·0
III	65·0	43·8	25·3
IV	34·2	33·5	11·3
V	30·3	22·6	15·2
VI	37·9	49·6	14·1
Mean	36·09	33·43	17·52
s.e.m.	6·18	4·56	2·21

Table 4. In vitro—in vivo correlation for three formulations of doxantrazole.

In vivo parameters	Correlation coefficient, r: % simulated absorption at 1 h	2 h	3 h
Peak plasma concentration	0·832	0·818	0·785
AUC (plasma)	0·989	0·985	0·975
Total (urinary) recovery	>0·999	>0·999	0·997

For significance at $P = 0{\cdot}05$, r = 0·9969.
$P = 0{\cdot}10$, r = 0·9877.

percentage absorbed in vitro is statistically significant at 1, 2 and 3 h for urinary recovery and at 1 h for AUC. An example of the correlation for AUC is shown in Fig. 2. The plasma concentration correlation did not reach statistical significance. The values

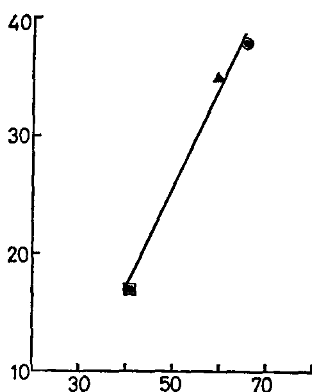

FIG. 2. Correlation of area under the plasma curve and simulated absorption data for three formulations of doxantrazole. ● = tablet, ▲ = suspension, ■ = solution. Ordinate: area under plasma curve μg ml^{-1} h. Abscissa: % simulated absorption at 1 h.

for total urinary excretions (Table 3) are for unchanged doxantrazole. Although these appear low (18–36%) previous studies in these laboratories have shown that the balance of the drug is in the faeces as a result of extensive biliary excretion. Hence doxantrazole absorption is essentially complete.

The unexpectedly poor absorption of this oral solution formulation is most probably due to the large volume of vehicle (100 ml) required to dissolve 200 mg of the drug. When taken orally the excipients may increase the lipophilicity of the gastrointestinal fluids and thus result in competition for the drug between the diluted fluids and the biological lipid barrier of the gastrointestinal tract. In addition a slower rate of diffusion through the gastrointestinal fluids may occur before absorption. These effects are shown most clearly in vitro with the artificial gastric barrier where the diffusion rate was reduced by a factor of twenty.

The fact that a good correlation was obtained in this study suggests that measurement of diffusion rates through artificial lipid membranes would be a useful predictive method for the evaluation of the availability of solid and liquid oral dosage forms.

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