International Journal of Pharmaceutics, 45 (1988) 123–128
Elsevier

IJP 01522

Theoretical model for interpretation of in situ absorption studies

J.B. Fariña, C.M. Evora, C.D. Espino and M. Llabrés

Departamento de Ingeniería Química y Tecnología Farmaceútica, Facultad de Farmacia, Universidad de La Laguna, Tenerife (Spain)

(Received 15 September 1987)
(Modified version received 20 December 1987)
(Accepted 30 December 1987)

Key words: In situ absorption study; Recirculation model; Mixed tank model; Statistical estimation; Non-linear regression; Sulphanilamide

Summary

A theoretical model was devised for the interpretation of in situ absorption studies using a recirculation system. The model, based on first-order kinetics and on the mixed tank model for intestinal circulation, showed the dependence of the apparent rate absorption constant upon the flow, reservoir volume, intestinal volume and absorption rate constant. The suitability of absorption clearance vs absorption rate constant for recirculation systems was concluded from statistical considerations. The model was used for the interpretation of sulphanilamide in situ absorption using male rats; the mean absorption clearance was 31.2 ± 5.80 ml/h.

Introduction

Ever since the introduction of the Doluisio method (1969), the in situ methods for the study of intestinal absorption of drugs has been widely accepted, and several modifications have been introduced into the experimental system used to perfuse the drug solution through the intestine (Schurgers et al., 1985; Houston and Wood, 1980). For the interpretation of the process of absorption properly considered, i.e., the passage of the drug from the intestinal lumen to the blood which irrigates the mucous membranes, two types of models are used: those which assume an absorption rate related to the whole amount or concentration of drug in the intestinal lumen, using a linear or non-linear model and those which include interpretation of the radial and/or axial diffusion of the drug in the intestinal lumen as well as the diffusion throughout the membrane (Amidon et al., 1980a and b; Winne, 1978, 1979).

In this article we propose a theoretical model to interpret the curves of concentration of remaining drug in the reservoir of the recirculation systems taking absorption to be a first-order process. To check out the model we have used sulphanilamide as test substance.

Theory

The theoretical model was based on the analysis of the scheme shown in Fig. 1 setting out the experimental procedure. Its development was divided into two parts: material balance and model of intestinal circulation.

Correspondence: J.B. Fariña, Departamento de Ingeniería Química y Tecnología Farmaceútica, Facultad de Farmacia, Universidad de la Laguna, Tenerife, Spain.

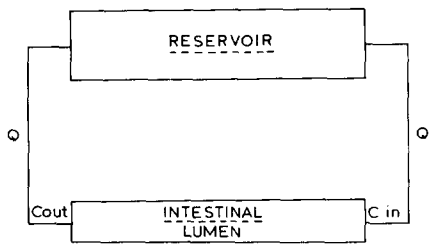

Fig. 1. Scheme of recirculation model used. Q, perfusion rate; C in and C out, intestinal input and output concentrations.

Applying the principle of material balance results in

$$V_R \frac{dc_{in}}{dt} = Q \cdot (c_{out} - c_{in}) \quad (1)$$

$$V_I \frac{d\bar{c}_1}{dt} = Q \cdot (c_{in} - c_{out}) - f(\bar{c}_1) \quad (2)$$

where V_R and V_I are the volumes of reservoir and intestinal lumen, respectively, Q, the perfusion rate, c_{in} and c_{out}, the intestinal input and output concentrations, $\bar{c}_1$, the mean luminal concentration (to be defined later) and f, the clearance function defining the absorption rate. Note that, due to the stirring action in the reservoir, the drug concentration within is equal to c_{in}.

The most extreme models used to characterize the perfusion liquid circulation mode inside the intestine are perfectly mixed model and plug flow. Himmelblau and Bischoff (1968) show that the concentration at the outlet from a system fed by a steady flow is expressed as follows:

$$c_{out} = \int_0^\infty c_e E(t) \cdot dt \quad (3)$$

where c_e is the concentration in each of the infinite elements into which the hypothetical system, in this case, the rat intestine, can be divided and in each of which a perfect mix may be assumed, while $E(t)$ is the output age distribution. Assuming that the absorption process may be interpreted according to first order kinetics, the concentration in any one element is reduced in accordance with the exponential equation:

$$c_e = c_{e,0}\, e^{-k_a t} \quad (4)$$

where k_a is the absorption rate constant. Combining Eqns. 3 and 4 gives

$$c_e = c_{e,0} \int_0^\infty e^{-k_a t} E(t)\, dt \quad (5)$$

The output age distribution in the perfectly mixed and plug flow models is expressed as follows (Himmelblau and Bischoff, 1968):

$$E(t) = (1/\bar{t}) \cdot e^{t/\bar{t}} \quad (6)$$

$$E(t) = \delta(t - \bar{t}) \quad (7)$$

where $\bar{t}$ is the intestinal mean residence time and δ, the Dirac impulse function. Entering these factors into Eqn. 5 results in:

$$c_{out}/c_{in} = 1/(1 + k_a \cdot \bar{t}) \quad (8)$$

$$c_{out}/c_{in} = e^{-k_a \bar{t}} \quad (9)$$

for perfectly mixed and plug flow models, respectively. The two equations are equivalent if $k_a \cdot \bar{t} < 0.20$ ($\epsilon \leq 0.021$ and $c_{out}/c_{in} > 0.80$ approximately) (i.e., $f(x)$ and $g(x)$ are said to be equivalent in the interval (x_a, x_b) if $f(x)/g(x) \leq 1 + \epsilon$, $\epsilon \to 0$) (Pisot and Zamansky, 1967). Hence, when the perfusion flow in the intestine is high in relation to its volume and thus $\bar{t}$ and the concentration gradient throughout the intestine are slight, the perfectly mixed model can be employed with marginal error, considerably simplifying the model.

Once the model of a perfectly mixed tank for the intestine has been selected, in this case $\bar{c}_1 = c_{out}$, the system formed by Eqns. 1 and 2 can be integrated. The initial conditions are that $t = 0$, $c_{in} = c_{in,0} = x_0/V_R$ and $c_{out} = 0$, where x_0 is the amount of dissolved drug in the reservoir; the resolution is:

$$c_{in} = (c_{in,0}/\lambda_1 - \lambda_2)(\lambda_1 - k_a - k_2)\, e^{-\lambda_1 t} + (k + k_2 - \lambda_2)\, e^{-\lambda_2 t} \quad (10)$$

$$c_{out} = (c_{in,0} \cdot k_a/\lambda_1 - \lambda_2)(e^{\lambda 2 t} - e^{\lambda 1 t}) \quad (11)$$

where

$$\lambda_1 = \left[(k_1 + k_2 + k_a) - \left(\sqrt{(k_1 + k_2 + k_a)^2 - 4k_1 \cdot k_a}\right)\right]/2 \quad (12)$$

$$\lambda_2 = \left[(k_1 + k_2 + k_a) + \left(\sqrt{(k_1 + k_2 + k_a)^2 - 4k_1 \cdot k_a}\right)\right]/2 \quad (13)$$

and

$$\lambda_1 + \lambda_2 = k_1 + k_2 + k_a \quad (14)$$

$$\lambda_1 \cdot \lambda_2 = k_1 \cdot k_a \quad (15)$$

For the graphic estimation of the parameters of the model, k_a and V_I, λ_1 and λ_2 were estimated by the stripping technique; as V_R, Q and x_o are known, k_a and V_I can be calculated, using Eqns. 14 and 15. For the final estimation of k_a and V_I, the non-linear regression programme BMDPAR (Ralston, 1981) was employed.

In accordance with Eqn. 10, it was expected that the drug concentration in the reservoir would decrease exponentially with time, after the lapse of a certain period, necessary for the pseudo-equilibrium between the two compartments, reservoir and intestine, to be reached, a phenomenon observed by other authors (Vranckx, 1979) as well as ourselves. The apparent rate constant in the model, λ_2, is a relatively complex function of the independent variables of the model: V_I, V_R, k_a and Q (Eqn. 13).

The graphs in Fig. 2 were made in order to study the relationship between λ_2 and the other variables, with values within the range found experimentally. The most important conclusions to be drawn are:

Firstly, λ_2 depends quasi-linearly upon $1/V_R$ (see Fig. 2) although it demonstrates asymptotic behaviour for very low values, but this has no practical interest. The greater k_a is, the more significant is this dependence; asymptotically for

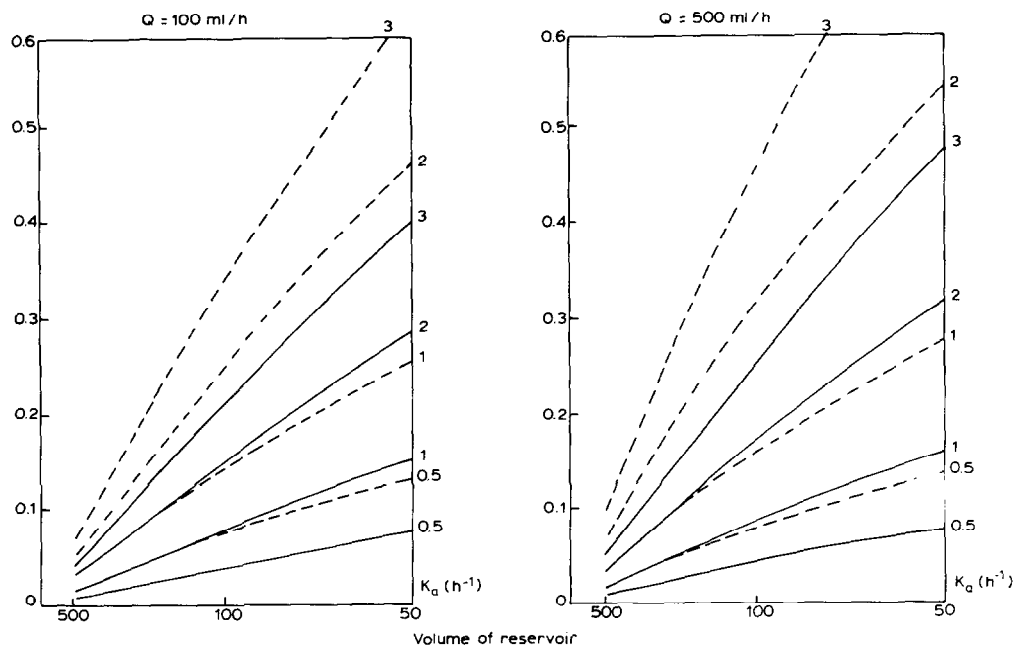

Fig. 2. Dependence of apparent absorption rate (λ_2) of flow (Q), volume of reservoir, absorption rate constant (K_a) and volume of intestinal lumen; (———), $V_I = 10$ ml; (------), $V_I = 20$ ml.

$k_a \to \infty$, $\lambda_2 \to Q/V_R$, the inverse mean residence time in the reservoir. However, there is another factor to complicate this interpretation, V_I. According to Eqn. 2, the absorption process is to be interpreted spatially, i.e., it is considered as taking place throughout the intestinal lumen, which in the model takes the place of a well-stirred reactor, and not merely on the wall; hence, the absorption rate is equal to $Cl_a \cdot \bar{c}_1$, where Cl_a is the absorption clearance. As Cl_a is also equal to $k_a \cdot V_I$, if the V_I is increased, the absorption rate is increased in the same proportion, as is the mean intestinal residence time (V_I/Q); however, the variations of k_a and V_I do not affect λ_2 in equal measure.

Secondly, the influence of the flow upon λ_2 depends on the absorption rate constant; the higher this is, the closer the dependence.

Thirdly, as mentioned above, k_a and V_I have a similar, but not equal, effect upon λ_2. In an experiment, V_R and Q can be fixed, and k_a and V_I estimated, adapting Eqn. 14 to experimental points by non-linear regression. As the phase of pseudoequilibrium, defined by λ_2, is extended for most of the time the experiment lasts, the correlation coefficient is most likely to be close to -1 between the estimates for V_I and k_a. This has important implications from the statistical estimation point of view; the surface of the sum of mean squares residuals will be banana-shaped and the S.D.s of the estimated values will be relatively high, as will be the dispersion among those estimated for different experiments. These forecasts were confirmed experimentally and constitute, in our opinion, the chief drawback to the use of recirculation methods for the study of in situ absorption.

Materials and Methods

Materials. A solution of sulphanilamide (2.90 mM) in a pH 6.20 buffer isotonized with sodium chloride. All other chemicals were analytical grade quality.

Test animals. Male albino Sprague–Dawley rats were used in the test. They were kept fasting for 19–20 h prior to the experiment. Water was given freely.

Rat in situ intestinal absorption. The apparatus for the recirculating technique consisted of a vessel thermostatically controlled at 37 °C containing 50 ml of sulphanilamide solution (2.90 mM, pH 6.20 at time zero) to be perfused while stirred; and a peristaltic pump producing a flow of 8.7 ml/min. The rats were anaesthetized with sodium pentobarbital 40 mg/kg i.p. A midline abdominal incision was made, and the small intestine was exposed. Glass tubings connected to silicone tubing were cannulated into the ends of the small intestine. Double ligations were made, taking care not to interfere with blood flow into the intestinal lumen during the experiments. The small intestine was washed with 50 ml of a perfusion solution (pH 6.20). Then the desired volume for the perfusion solution containing the antibiotic was recirculated from the duodenal to the ileum.

Analysis. The sulphanilamide was determined by the Bratton–Marshall (1939) spectrophotometric technique.

Results and Discussion

Table 1 sets out the concentration of sulphanilamide remaining in the reservoir in the 3 experiments carried out. Table 2 summarizes,

TABLE 1

Concentrations of sulphanilamide remaining in the reservoir (μg/ml) for each of 3 experiments

Hours	1	2	3
0.000	502	486	472
0.042	369	385	385
0.083	335	376	383
0.125	329	370	375
0.167	317	363	372
0.250	302	360	365
0.375	297	335	329
0.500	283	309	313
0.750	247	300	312
1.00	208	245	268
1.25	183	222	245
1.50	167	190	216
1.75	144	185	187

TABLE 2

Results of parameter estimation and runs of residuals test

Exp.	$k_a \pm$ S.D. (h^{-1})	$V_I \pm$ S.D. (ml)	r	Cl $\pm$ S.D. (ml/h)	f
1	1.53 ± 0.093	24.5 ± 0.923	-0.879	37.6 ± 2.09	0.733
2	1.98 ± 0.187	15.1 ± 0.977	-0.914	29.9 ± 2.59	0.471
3	1.77 ± 0.216	14.8 ± 1.19	-0.909	26.2 ± 2.93	0.121

Exp., experiment number; k_a, absorption rate constant; V_I, volume of intestinal lumen; r, correlation coefficient of estimates; Cl, absorption clearance; f, null hypothesis probability for runs of residuals test.

among other findings, the k_a and V_I estimated by non-linear regression. To check the validity of the theoretical model proposed, the residual signs test (Draper and Smith, 1966) was applied; the type α, probability values per test are given in the fifth column of Table 2; the adjustment was satisfactory. As theorized, the correlation coefficient between the estimates for k_a and V_I is invariably close to 1 which makes the estimation of both parameters difficult and indirectly provokes an increase in interexperimental variability. To overcome this problem we propose using absorption clearance equal to $k_a V_I$; the estimation variance of this new parameter can be assessed approximately by expressing it as a linear combination of the primitive parameter variance via the Taylor theorem, i.e.:

$$\mathrm{VAR(Cl)} \simeq (\partial \mathrm{Cl}/\partial k_a)^2 \mathrm{VAR}(k_a) + (\partial \mathrm{Cl}/\partial V_I)^2 \mathrm{VAR}(V_I) \qquad (16)$$

The result of the experiments were set out in Table 2, with a mean value of 31.2 ± 5.80 ml/min.

This model is totally unlike that of Tsuji et al. (1978) which did not interpret the passage of the drug from the reservoir to the intestine but assumed the absorption rate to be in proportion to the full amount of drug present in the reservoir; the apparent absorption rate constant, λ_2 in our model, seemed independent of the flow, although the experimental evidence had suggested the contrary. Furthermore, the test given by Tsuji et al. (1978) to check their model, the linear dependence of the apparent absorption rate constant of the inverse volume of the reservoir, can be accounted for by our model without recourse to the degradation of the active principle, as can be seen from Fig. 2. In short, our model enables the effects of the experimental variables, perfusion flow through the intestine and reservoir volume, to be interpreted correctly, with the following conditions: the volume of the flow should be such that the intestine can be viewed from the material balance point as a "well mixed tank" and the absorption process should be linear and not limited by radial diffusion. The problem incurred by the high correlation to be expected of the estimates of k_a and V_I can be relieved by using their product, absorption clearance.

We saw earlier that the use of a relatively high perfusion flow enabled us to interpret the in situ intestinal absorption as an irreversible first-order-kinetics process with a well-mixed tank. However, the flow customarily employed in this type of study is sufficiently low to presuppose that the type of fluid circulation will be laminar, a fact confirmed experimentally by Amidon et al. (1980b) whereby the influence of the perfusion flow is not limited to the mean intestinal residence time but may also affect the axial dispersion of the drug. Furthermore, although the absorption takes place on the surface of the intestinal epithelium, the model assumes a homogeneous process in all the intestinal lumen, and did not assess the possible influence of the perfusate flow upon the radial diffusion of the drug. Hence, the model proposed is only useful for comparison purposes under certain experimental conditions and for the reasons outlined in the previous paragraph, absorption clearance should be used to quantify the absorption rate in situ.

Acknowledgements

This work has been financed by the Comisión Asesora de Investigación Científica y Técnica of Spain (Project 1792-82).

References

Amidon, G.L., Leesman, G.D. and Elliot, R.L., Improving intestinal absorption of water-insoluble compounds: a membrane metabolism strategy. *J. Pharm. Sci.*, 69 (1980a) 1363–1368.

Amidon, G.L., Kou, J., Elliot, R.L. and Lightfoot, E.N., Analysis of models for determining intestinal wall permeabilities. *J. Pharm. Sci.*, 69 (1980b) 1369–1373.

Bratton, A.C. and Marshall, E.K., A new coupling component for sulfamide determination. *J. Biol. Chem.*, 128 (1939) 537–550.

Doluisio, J., Billups, N.F., Dittert, L.W., Sugita, F. and Swintoski, J.V., Drug absorption I. An in situ rat technique yielding realistic absorption rates. *J. Pharm. Sci.*, 58 (1969) 1196–1200.

Draper, N.R. and Smith, H., The examination of residuals. In *Applied Regression Analysis*, Wiley, New York, 1966, pp. 98–99.

Himmelblau, D.M. and Bischoff, K.B., Population - balance models. In *Process Analysis and Simulation: Deterministic Systems*, Wiley, New York, 1968, pp. 64–184.

Houston, J.B. and Wood, S.G., Gastrointestinal absorption of drugs and other xenobiotics. In Bridges, J.N. and Chausseaud, L.T. (Eds.), *Progress in Drug Metabolism, Vol. 4*, Wiley, New York, 1980, pp. 60–75.

Pisot, C. and Zamansky, M., Estudio local de las funciones numericas. In *Matematicas Generales. Algebra/Analisis*. Montaner y Simon, Barcelona, 1967, pp. 494–497.

Ralston, M., Derivate-free non linear regression. In Dixon N.J. and Brown, M.B. (Eds.), *Biomedical Computer Programs*. University of California Press, Berkeley, 1981, pp. 305–314.

Schurgers, N., Bijdendijk, J., Tukker, J.J. and Crommelin, D.J.A., Comparison of four experimental techniques for studying drug absorption kinetics in the anesthetized rat in situ. *J. Pharm. Sci.*, 75 (1985) 117–119.

Tsuji, A., Miyamoto, E., Kagami, I., Sakaguchi, H. and Yamana, T., G.I. Absorption of β-lactam antibiotics, I. Kinetic assessment of competing absorption and degradation in G.I. tract, *J. Pharm. Sci.*, 67 (1978) 1701–1704.

Vranck, H., Metodologia nueva para apreciar la biodisponibilidad de los medicamentos: difusiòn in vivo y resorciòn in situ. In *I Congreso Hispano Frances de Biofarmacia y Farmacocinètica, Vol. 2*, Concejo Oficial de Colegios de Farmaceuticos, Madrid, 1979, pp. 425–438.

Winne, D., Dependence of intestinal absorption in vivo on the unstirred layer. *Arch. Pharmacol.*, 304 (1978) 175–181.

Winne, D., Rat jejunum perfused in situ effect of perfussion rate and intraluminal radius on absorption rate and effective unstirred layer thickness. *Arch. Pharmacol.*, 307 (1979) 265–274.