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European Journal of Pharmaceutics and Biopharmaceutics 52 (2001) 31–37

European Journal of Pharmaceutics and Biopharmaceutics

www.elsevier.com/locate/ejphabio

Research paper

The influence of active secretion processes on intestinal absorption of salbutamol in the rat

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Received 6 June 2000; accepted in revised form 27 March 2001

Abstract

Salbutamol was perfused in the small intestine of rat using a standard rat gut 'in situ' preparation: (1) in inhibitor-free solution at seven different concentrations (0.15, 0.29, 1.20, 5.0, 9.0, 13.0 and 18.0 mM); (2) at a 0.29 mM concentration – thought to be close to the allometric dose in man – in the presence of a non-specific enzyme inhibitor, sodium azide (0.3, 3.0 and 6.0 mM); and (3) at 0.29 mM in the presence of a selective secretion inhibitor, verapamil (10.0 and 20.0 mM). In free solution, the mixed-order rate constants, k'_a, of salbutamol increase as the solute concentration increases until an apparent asymptotic value is reached. This could be due to the saturation of enzymatic systems responsible for the secretion of the drug from the enterocyte to the luminal fluid, a process that could explain the poor absorption of salbutamol. In the presence of sodium azide, the k_a values increased about 1.5-fold, whereas in the presence of verapamil they increased two- to three-fold. These results indicate that salbutamol can act as a substrate of an intestinal secretory transport, which probably includes – at least in part – the enzyme P-glycoprotein, since verapamil has been shown to inhibit this enzyme by dose-dependent competition. This leads to a secretion-limited peroral absorption of salbutamol, which contributes to the poor oral bioavailability of the drug. The possible options for improving salbutamol absorption are discussed.

Keywords: Salbutamol; Intestinal absorption; Secretion; P-glycoprotein; Dose-dependent inhibition of secretion

1. Introduction

Salbutamol is a beta-2-adrenergic agonist that has been widely used in the treatment of asthmatic disorders and chronic obstructive lung diseases. Its absolute peroral bioavailability has been shown to be scanty and variable (10–50% in humans [1,2]), and this has been attributed to a first-pass intestinal or hepatic metabolism or merely to a poor intestinal permeation capacity [3]. However, it could also be due – at least in part – to an active secretion of the drug from the enterocytes into the intestinal luminal content. In the past, excretion of drugs by the intestine was believed to occur mainly via the biliary route, but several recent reports indicate that excretion into the luminal fluid can be an important route for the elimination of certain drugs. The presence of specific transporters of drugs from the membrane and even the cytoplasm of enterocytes to the luminal fluid has been demonstrated. One of the secretory transporters is P-glycoprotein, which functions as an efflux transport pump for a variety of drugs, including certain antimitotic agents; it has been identified in the brush-border membrane of the intestinal mucosa [4]. Nevertheless, the negative influence of these excretion phenomena on the apparent absorption rate of the drug – as presystemic losses – becomes evident.

Beta-blockers such as acebutolol, celiprolol, nadolol, timolol, and others are actively secreted into the luminal fluid [5,6], and the oral bioavailability of these drugs is thereby greatly reduced. On the other hand, these active excretion phenomena are inhibited by a number of drugs that are substrates of the corresponding enzymes, such as vinblastine, nifedipine, and particularly verapamil [7,8]. Although salbutamol is a beta-agonist drug possessing completely different pharmacological actions, it presents clear chemical similarities to the above beta-blockers. It is therefore not risky to postulate that it could also be a substrate for active secretion and, consequently, subject to bioavailability problems.

In the present study, an attempt is made to elucidate whether the poor peroral bioavailability of salbutamol is related, at least in part, to the above-indicated active excretion phenomena. For this purpose, we have studied the intestinal absorption of the drug: (a) in free solution, at

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0939-6411/01/$ - see front matter
PII: S0939-6411(01)00155-2

different concentrations in the intestinal perfusion fluid; (b) in the presence of a non-specific metabolic inhibitor, as is the case of the sodium azide; and (c) in the presence of the specific enzymatic secretion inhibitor verapamil.

Experimental studies concerning the effects of these enzymes, particularly P-glycoprotein, on drug absorption are usually carried out on monolayer cell cultures such as Caco-2 cells [6,7]. In our case, the experiments were carried out in anesthetized animals with the aid of the in situ rat gut perfusion technique [9–12], which normally provides very realistic absorption rates. Appropriate tests were previously performed in order to determine the chemical stability of salbutamol in the working intestinal fluid during the experiments, to be sure that the drug disappearance from the luminal content was only due to genuine absorption phenomena.

2. Materials and methods

2.1. Degradation studies

Usual perfusion solutions, buffered to pH 6.4 in order to reproduce the in situ absorption tests: (a) as such and (b) without salbutamol (Laboratorios Aldo-Unión, Barcelona, Spain) but with the highest verapamil (Sigma–Aldrich–Química S.A. Madrid, Spain) or sodium azide (Sigma–Aldrich–Química S.A. Madrid, Spain) concentration used (20 or 6 mM, respectively) were prepared. Aliquots of 10 ml of each solution were perfused along the whole length of the small intestine according to the general absorption technique, as described later, for a period of 30 min (four animals per series). After perfusion, each solution was recovered, as indicated for water reabsorption tests, filtered through Whatman 1 paper (Vidra Foc S.A. Barcelona, Spain), and diluted with an isotonic solution containing: (a) 5.8 mM of salbutamol or (b) 20 mM of verapamil or 6 mM of sodium azide and 5.8 mM of salbutamol (l9/1, v/v), in order to obtain the same concentrations used in the absorption tests. Four aliquots of each solution were placed in Eppendorff tubes. One of the aliquots was used as the reference standard and frozen at −20°C until quantification. The remaining aliquots were incubated at 37°C in a water bath for 10, 20, and 30 min, and each sample was immediately tested for the salbutamol content by the HPLC method, which will be described later, using a freshly prepared solution of salbutamol.

2.2. Absorption tests

The animal experimentation was approved by the Research Commission of the Department Council. All absorption studies were performed on male Wistar rats weighing 250–300 g, fasted for 24 h but with free access to water. Anesthesia was induced 1 h before surgery by a 25% (m/v) intraperitoneal injection of ethyl urethane (Scharlab, Barcelona, Spain) 0.4 ml per 100 g of animal weight. The in situ rat gut technique [9], modified as previously reported [10,12], was used.

A volume of 10 ml of 0.15, 0.29 (corresponding to the allometric dose relative to man [13]), 1.20, 5.0, 9.0, 13.0, or 18.0 mM isotonized salbutamol solutions was perfused in the whole small intestine at 37°C ($n = 8$) in the first series of experiments. For the second series, 10 ml of a 0.29 mM salbutamol solution in the presence of 0.3, 3.0, or 6.0 mM of sodium azide was isotonized and perfused in the whole small intestine ($n = 8$). Finally, in a third series of assays 10 ml of a 0.29 mM salbutamol solution in the presence of 10 or 20 mM of verapamil was isotonized and perfused as above ($n = 8$).

Water reabsorption during the experiment was rather scanty (between 6.2 and 8.7%). Notwithstanding, it was evaluated and corrected separately for each animal using a direct volumetric procedure already described [12,14].

2.3. Analytical procedures

Intestinal samples were assayed for salbutamol content by HPLC fluorometry, with excitation and emission wavelengths of 229 and 306 nm, respectively. A Hewlett–Packard apparatus made up of an HP 1050 pump, an HP 1046A fluorescence detector, and an HP 3395 integrator was used. A Model 7125 Rheodyne injector with a 100 μl loop size was employed for application of samples (Hewlett–Packard, Barcelona, Spain). As stationary phase, a Spherisorb S5-ODS2 (150 × 4.6 mm) column (Waters, Barcelona, Spain), together with a C-130B (Teknokroma C-18, Teknokroma S.L. Barcelona, Spain) precolumn, was utilized. As mobile phase, a mixture of methanol (Scharlab) and a 30 mM solution of orthophosphoric acid (Scharlab) (pH 4.0) in volumetric proportions of 21/79 was used at a flux of 1.0 ml min^{-1}. Samples were centrifuged at 3000 rpm for 10 min, and 20.0 μl of the supernatant was injected into the chromatograph.

Calibration curves covering the entire range of the salbutamol concentrations were prepared. Excellent linear plots relating the peak areas and the salbutamol concentrations were obtained. The accuracy and precision of the method were validated. The criteria were assessed using four salbutamol concentrations in the absence and in the presence of verapamil or sodium azide, covering the entire calibration range of the analytical method. The accuracy was evaluated by calculating the relative error, which was always less than 3.33%. The precision was assessed by calculating the coefficient of variation, which in no case was higher than 3.45%. These features were believed to be completely acceptable from a biopharmaceutical viewpoint [15].

2.4. Absorption rate measurements

Intestinal absorption of salbutamol was quantified using its apparent first-order rate constants as usual, according to the classic expression:

Table 1
Results of the degradation tests and statistical analysis of the differences (two-way ANOVA test)

Condition	*n*	Time (min)	Salbutamol (mM)		Statistical analysis		
					F	*P*	Significance
Salbutamol 0.29 mM (free solution)	4	10	0.292 (±0.003)	Time	1.042	0.365	NS
	4	20	0.288 (±0.001)				
	4	30	0.291 (±0.005)				
Salbutamol 0.29 mM + sodium azide 6 mM	4	10	0.289 (±0.003)	Condition	1.092	0.348	NS
	4	20	0.288 (±0.005)				
	4	30	0.287 (±0.006)				
Salbutamol 0.29 mM + verapamil 20 mM	4	10	0.293 (±0.005)				
	4	20	0.291 (±0.002)				
	4	30	0.292 (±0.003)				

$$A = A_0\, e^{-k'_a t} \quad (1)$$

where A represents the concentrations of salbutamol remaining in the luminal fluid already corrected for water reabsorption at the sampling times, t, and A_0 is the calculated intercept at zero time, which is usually lower than the initial concentration perfused due to initial membrane adsorption of the solute and/or to sample dilution [12,16]. In order to overcome these effects, only the samples obtained between 5 and 30 min were used for calculations, i.e. the initial non-perfused sample was excluded. On the other hand, k'_a is the apparent absorption rate constant, which depends on the remaining concentration of salbutamol in the intestinal lumen, which in turn depends simultaneously on the instantaneous losses in absorption rate and the instantaneous luminal secretion gains. It is, therefore, an apparent mixed-order rate constant that can be mathematically expressed, as pointed out and explained in Appendix A.

Both parameters – A_0 and k'_a – were then calculated for each individual animal according to a non-linear least-squares regression procedure, using the PC NONLIN program (Simplex algorithm). The correlation coefficients, r, between theoretically predicted and experimentally obtained values were used to assess the goodness-of-fit. The k'_a individual values found for each data set were statistically compared using a one-way analysis of variance (ANOVA) test. Results were assumed to be significant for a 95% probability value ($P < 0.05$).

3. Results and discussion

3.1. Degradation assays

In degradation assays, the salbutamol concentration in the luminal samples was determined after incubation at 37°C for different lengths of time both in the absence and in the presence of inhibitors. Results and statistical analysis (ANOVA) of the data are given in Table 1. As can be seen, if degradation of salbutamol (chemical or microbial) occurs, it is not statistically significant. Therefore, it can be assumed that under our experimental conditions, the disappearance of salbutamol from the intestinal fluid is exclusively due to an actual absorption process. In other words, we did not find chemical interactions of salbutamol with intestinal luminal fluid components or with intestinal bacterial flora that could influence the experimentally determined apparent absorption rate constant.

3.2. Solute concentration effects

The in situ apparent absorption rate constants (k'_a, h^{-1})

Table 2
Absorption rate constants (±SD) found in rat intestine for different salbutamol concentrations and statistical analysis of differences (one-way ANOVA test)

	Concentrations (mM)	k'_a (h^{-1}) (±SD)	*n*	Statistical analysis		
				F	*P*	Significance
Salbutamol solutions (pH 6.4)	0.15	0.281 (±0.112)	8	21.540	< 0.0001	S
	0.29	0.420 (±0.05)	8			
	1.20	0.576 (±0.04)	8			
	5.00	0.635 (±0.03)	8			
	9.00	0.695 (±0.09)	8			
	13.00	0.611 (±0.08)	8			
	18.00	0.635 (±0.04)	8			

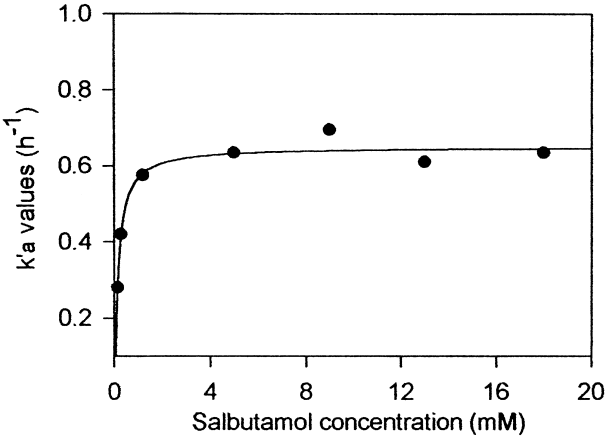

Fig. 1. Plot of the fit relating apparent k'_a values, non-linear in nature, and salbutamol concentration in the perfusion fluid. Equation parameters are shown in Appendix A.

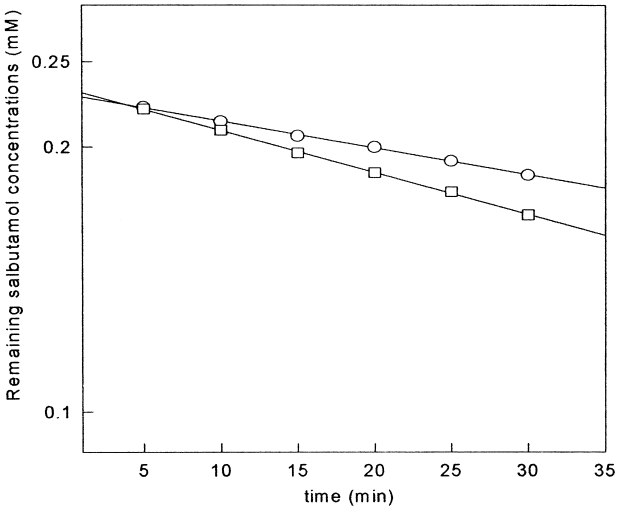

Fig. 2. Plot of the fit of the apparent first-order equation to the data (remaining luminal concentrations of salbutamol when initial sodium azide concentrations are 0 (○) and 6 mM (□)).

obtained for salbutamol in free solution at the seven selected concentrations (mM) are given in Table 2. The significance of the differences between the constants found at each selected concentration by means of a one-way ANOVA test is also specified. As can be observed, the k'_a values significantly increase as the salbutamol concentration in the working fluid increases; the gain, however, is progressively lower until an apparently asymptotic value is reached, as shown in Fig. 1.

These results can be tentatively interpreted by assuming that when the salbutamol concentration at its absorption site (i.e. in the membrane and the cytoplasm of the enterocyte) progressively increases, a saturation of the active secretory system is reached. In these conditions, the losses due to excretion are proportionally lower and the absorption yield improves. As a result, a progressively higher apparent absorption rate constant is obtained when the concentration of salbutamol in the working fluid is increased. When it is so high that the excretion system becomes completely saturated, k'_a stabilizes at a constant value, i.e. $0.649 \pm 0.016\ h^{-1}$, as we attempt to explain in mathematical terms in Appendix A. These considerations are consistent with the data shown in Table 2, which effectively lead to this asymptotic k_a value.

3.3. Influence of the enzyme inhibitors

3.3.1. Non-specific inhibitors

Sodium azide, which is a non-specific enzyme inhibitor [18], influences salbutamol absorption in a 0.29 mM solution at mM concentrations of 0.3, 3.0, and 6.0, as can be seen in Table 3 and in Fig. 2. The increases in the k'_a values as the concentration of azide in the perfusion fluid increases are moderately marked. From the ANOVA test, however, it can be observed that there are significant differences between azide-containing solutions and the free salbutamol one, and this means that the salbutamol fraction that has reached the enterocyte can be excreted to the luminal fluid by means of an energy-dependent mechanism, which is inhibited by sodium azide. Note that the action of this non-specific inhibitor appears even at a very low concentration in the perfusion fluid (0.30 mM, like that of salbutamol) and does not substantially increase when the concentration increases.

3.3.2. Specific competitive inhibitors

The in situ k'_a values obtained for salbutamol at the

Table 3
Absorption rate constants (±SD) observed in rat intestine for free salbutamol in the absence and in the presence of different concentrations of inhibitor and statistical analysis of differences (one-way ANOVA test)

Condition	Concentration of inhibitor (mM)	n	k'_a (h^{-1}) (±SD)	Statistical analysis		
				F	P	Significance
Salbutamol 0.29 mM with sodium azide	0	8	0.42 (±0.05)	36.639	< 0.0001	S
	0.3	8	0.59 (±0.03)			
	3	8	0.61 (±0.05)			
	6	8	0.64 (±0.05)			
Salbutamol 0.29 mM with verapamil	0	8	0.42 (±0.05)	284.533	< 0.0001	S
	10	8	0.99 (±0.08)			
	20	8	1.23 (±0.07)			

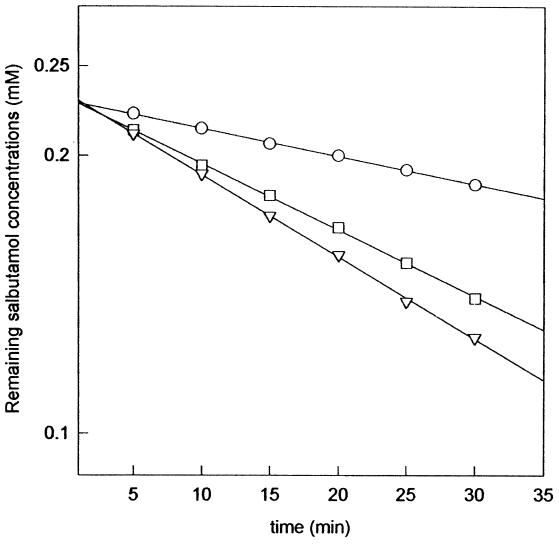

Fig. 3. Plot of the fit of the apparent first-order equation to the data (remaining luminal concentrations of salbutamol when initial verapamil concentrations are 0 (○), 10 (□), and 20 mM (▽)).

0.29 mM concentration in the presence of the specific enzyme competitive inhibitor verapamil are also detailed in Table 3.

Verapamil can compete with some drugs for active excretion via P-glycoprotein and/or other enzymatic systems [8,17,19]. If the competition actually exists, addition of verapamil to the perfusion fluid should provide a better, more complete absorption of salbutamol. The one-way ANOVA test clearly demonstrates highly statistically significant differences between the k'_a values of salbutamol in free solution and those obtained in the presence of verapamil at both concentrations assayed (10.0 and 20.0 mM). In fact, the k'_a values are 2.4 and 3.0 times greater, respectively, than those obtained in free solution, thus demonstrating a dose-dependent inhibition. In Fig. 3 it can be clearly seen that the slope of the remaining drug concentration/time lines tends to increase as the verapamil concentration in the working fluid is increased.

As pointed out above, verapamil has been assumed to be a competitive enzyme (including P-glycoprotein) secretion inhibitor, but the k'_a values found for salbutamol in the presence of verapamil at the two concentrations used are clearly greater than the asymptotic k'_a value found for the drug in free solution (Table 2) or in 0.29 mM solution in the presence of 6.0 mM sodium azide (Table 3). This could lead to the conclusion that there is another absorption-promoting factor that is not easy to elucidate.

Apart from the blocking action of verapamil on the calcium channels, which can lead to alterations in tight-junction formation with increased penetration of salbutamol and other low molecular weight compounds through the paracellular route, verapamil is a strong vasodilator, and since very high concentrations were co-perfused with salbutamol, mesenteric circulation may have significantly increased and provoked increases in the absorption rates of salbutamol as well. In order to gain an insight into this question, we studied the absorption rate of salbutamol coadministered with antipyrine (which is not subject to intestinal secretion and has a molecular weight very suitable for these purposes). The results, shown in Table 4, seem to indicate that these possible subsidiary effects are not conclusive, at least to a significant degree.

Work based on the use of a more selective competitive P-glycoprotein inhibitor [20] is in progress to clarify this point. We are also developing monolayer cell cultures (Caco-2 cells overexpressed in P-glycoprotein) in order to compare results. Nevertheless, the inhibition of the action of the secretory enzyme systems can undoubtedly enhance the salbutamol absorption yield.

3.4. Biopharmaceutical implications

Salbutamol is a potent, widely used bronchodilator drug marketed in different pharmaceutical dosage forms [21]. For acute asthmatic crises, aerosol dosage forms are available, but their effects are brief and continuous use leads to excessive dryness of the bronchial mucosa. The transpulmonary route is not the best for chronic disorders, which are better dealt with via peroral administration. The use of the latter is, however, rather limited due to the poor bioavailability of salbutamol in the usual dosage forms.

It would, therefore, be interesting to find suitable solutions to these limitations in the use of salbutamol. One of them could obviously (though not easily) be the addition of a physiologically harmless specific enzyme inhibitor to the pharmaceutical peroral dosage forms of salbutamol. Perhaps a better solution would be to find an administration route that could elude the secretory effect that P-glycopro-

Table 4
Absorption rate constants (±SD) observed in rat intestine for free antipyrine in the absence and in the presence of different concentrations of inhibitor and statistical analysis of differences (one-way ANOVA test)

Condition	Concentration of inhibitor (mM)	n	k'_a (h^{-1}) (±SD)	Statistical analysis		
				F	P	Significance
Antipyrine 0.25 mg ml^{-1} with verapamil	0	8	2.92 (±0.24)	2.930	< 0.075	NS
	10	8	2.81 (±0.34)			
	20	8	2.67 (±0.17)			

tein and other enzymes exhibit in the gastrointestinal tract. For example, buccal or sublingual administration of bioadhesive forms a few hours before sleeping could provide effective, sustained blood concentrations of salbutamol, thus increasing the life quality of many patients. Therefore, we are presently engaged in research that is independent of the biological studies reported here and focuses on the biopharmaceutical design and technological preparation of bioadhesive buccal tablets of salbutamol, as a further part of this project.

4. Conclusions

The results obtained using different perfusion concentrations of salbutamol, as well as those found in the presence of non-specific (sodium azide) and a competitive specific (verapamil) inhibitors, clearly demonstrate that the active secretion of the drug from the enterocyte to the luminal fluid is a critical factor in the poor peroral bioavailability of the drug. This active secretion probably involves a P-glycoprotein enzyme type, as can be deduced from the inhibition studies, which show a 1.5- to three-fold increase in the apparent absorption rate constant of salbutamol, k'_a. Notwithstanding, other absorption-promoting mechanisms characteristic of the specific inhibitor cannot be ruled out.

Acknowledgements

This work is a part of the Project SAF 97-250, funded by the CICYT of the Ministry of Education and Culture of Spain. The authors are indebted to the University of Valencia for a B.V. grant. The authors are grateful to Laboratorios Aldo-Unión (Barcelona) for supplying salbutamol. They are also indebted to Professor Dr J.M. Plá-Delfina for his review and useful discussion of the manuscript and to Jane Heilker for reviewing and correcting the English text.

Appendix A

The amount of salbutamol that would permeate across the enterocyte per time unit, dA/dt, provided that a first-order kinetics existed, would be k_aA. Consequently, if there were active secretion of salbutamol from the enterocyte to luminal fluid, the global absorption rate would be:

$$-\frac{dA}{dt} = k_aA - \frac{V_mA}{K_m + A} \quad (A1)$$

where k_a is the maximal absorption rate constant of the drug that can be obtained under our working conditions, and V_m and K_m are constants governing the active secretion process. Rearranging this expression, we have:

$$-\frac{dA}{dt} = A\left(k_a - \frac{V_m}{K_m + A}\right) \quad (A2)$$

and:

$$-\frac{dA/dt}{A} = k_a - \frac{V_m}{K_m + A} \quad (A3)$$

By definition, $-(dA/dt)/A$ represents the apparent experimentally determined absorption rate constant, k'_a. That is:

$$k'_a = k_a - \frac{V_m}{K_m + A} \quad (A4)$$

As the amount of drug at the absorption site is increased, the quotient $V_m/(K_m + A)$ decreases, and when A is so great that the secretion becomes completely saturated, $V_m \lll (K_m + A)$, and the quotient tends to zero. Then, Eq. (A4) becomes:

$$k'_a \approx k_a \quad (A5)$$

In other words, the apparent absorption rate constant that is measured tends to be equal to the maximal k_a value of salbutamol that can be found in our working conditions (i.e. the asymptotic value). Moreover, V_m and K_m are functional or apparent constants since A represents the initial working concentration which, in fact, changes with time.

From the data of Table 2, graphically shown in Fig. 1, the following parameters and statistical figures were found: $V_m = 0.0860 \pm 0.024$ mM h^{-1}; $K_m = 0.0836 \pm 0.068$ mM; $k_a = 0.649 \pm 0.016$ h^{-1} (SS = 0.0094; $r = 0.984$), where r is the correlation coefficient between model-predicted and experimentally obtained k'_a values.

References

[1] M.J. Hutching, J.D. Paull, E.E. Wilson, D.J. Morgan, Pharmacokinetics and metabolism of salbutamol in premature labour, Br. J. Clin. Pharmacol. 24 (1) (1987) 69–75.
[2] A.H. Price, S.P. Clissold, Salbutamol in the 1980s: a reappraisal of its clinical efficacy, Drugs 38 (1) (1989) 77–112.
[3] A.M. Taburet, B. Schmit, Pharmacokinetic optimisation of asthma treatment, Clin. Pharmacokinet. 26 (5) (1994) 396–418.
[4] S. Hsing, Z. Gatmaitan, I.M. Arias, The function of Gpl70, the multidrug-resistance gen product, in the brush border of rat intestinal mucosa, Gastroenterology 102 (1992) 879–885.
[5] F. Thiebaut, T. Tsuruo, H. Hamada, M.M. Gottesman, I. Pastan, M.C Willingham, Cellular localization of the multidrug-resistance gen product P-glycoprotein in normal human tissues, Proc. Natl Acad. Sci. USA 84 (1987) 7735–7738.
[6] S.M. Kuo, B.R. Whitby, P. Arthursson, J.A. Ziemniak, The contribution of intestinal secretion to the dose-dependent absorption of celiprolol, Pharm. Res. 11 (1994) 648–653.
[7] S. Doppenschmitt, H. Spahn-Langguth, C.G. Regardh, P. Langguth, Radioligand-binding assay employing P-glycoprotein-overexpressing cells: testing drug affinities to the secretory intestinal multidrug transporter, Pharm. Res. 15 (7) (1998) 1001–1006.
[8] R. Sandstrom, A. Karlsson, L. Knutson, H. Lennernas, Jejunal absorption and metabolism of R/S-verapamil in humans, Pharm. Res. 15 (6) (1998) 856–862.
[9] J.T. Doluisio, N.F. Billups, L.W. Dittert, E.T. Sugita, J.V. Swintosky, Drug absorption. I. An in situ rat gut technique yielding realistic absorption rates, J. Pharm. Sci. 56 (1969) 1196–1200.
[10] A. Sánchez-Picó, J.E. Peris-Ribera, C. Toledano, F. Torres-Molina, V.G. Casabó, A. Martín-Villodre, J.M. Plá-Delfina, Nonlinear intest-

inal absorption kinetics of cefadroxil in the rat, J. Pharm. Pharmacol. 41 (1989) 179–185.

[11] J.M. Plá-Delfina, M.D. Pérez-Buendía, V.G. Casabó, J.E. Peris-Ribera, E. Sánchez-Moyano, A. Martín-Villodre, Absorption-partition relationships for true homologous series of xenobiotics as a possible approach to study mechanisms of surfactants on absorption. I. Aromatic amines in rat colon, Int. J. Pharm. 43 (1987) 49–64.

[12] A. Martín-Villodre, J.M. Plá-Delfina, J. Moreno-Dalmau, M.D. Pérez-Buendía, J. Miralles, E. Collado, E. Sánchez-Moyano, A. Del Pozo, Studies on the reliability of a bihyperbolic functional absorption model. I. Ring substituted anilines, J. Pharmacokinet. Biopharm. 14 (1986) 615–633.

[13] J.E. Peris-Ribera, F. Torres-Molina, M.C. García-Carbonell, M. Merino, J.M. Plá-Delfina, Absorción activa y alometría: posibles relaciones aplicables a los modelos de biodisponibilidad, Cien. Ind. Farm. 5 (1986) 282–288.

[14] V. Sancho-Chust, M. Bengochea, S. Fabra-Campos, V.G. Casabó, M.J. Martínez-Cámara, A. Martin-Villodre, Experimental studies on the influence of surfactants on intestinal absorption of drugs. Cefadroxil as model drug and sodium laurylsulfate as model surfactant: studies in rat duodenum, Arzneim. Forsch. 45 (1995) 1013–1017.

[15] H.T. Karnes, C. March, Precision, accuracy, and data acceptance criteria in biopharmaceutical analysis, Pharm. Res. 10 (1993) 1420–1426.

[16] J.T. Doluisio, W.G. Crouthamel, G.H. Tan, J.V. Swintosky, L.W. Ditter, Drug absorption. III. Effect of membrane storage on the kinetics of drug absorption, J. Pharm. Sci. 59 (1970) 72–76.

[17] H. Terao, E. Hisanaga, Y. Sai, Y. Tamai, A. Tsuji, Active secretion of drugs from the small intestinal epithelium in rats by P-glycoprotein functioning as an absorption barrier, J. Pharm. Pharmacol. 48 (10) (1996) 1083–1089.

[18] E. Estrada, M. Hernández, Portadores múltiples, in: H.M. Blume (Ed.), Cinética de transporte a través de membranas, Madrid, 1976, pp. 127–145.

[19] S. Doppenschmitt, P. Langguth, C.G. Regardh, T.B. Andersson, C. Hilgendorf, H. Spahn Langguth, Characterization of binding properties to human P-glycoprotein: development of a [H-3] verapamil radioligand-binding assay, J. Pharmacol. Exp. Ther. 288 (1) (1999) 348–357.

[20] S.P. Letrent, G.M. Pollack, K.R. Brouwer, K.L.R. Brouwer, Effect of GF120918, a potent P-glycoprotein inhibitor, on morphine pharmacokinetics in the rat, Pharm. Res. 15 (1998) 559–605.

[21] R.M. Hernández, A.R. Gascón, M.B. Calvo, C. Caramella, U. Conte, A. Domínguez-Gil, J.L. Pedraz, Influence of route of administration and dosage form in the pharmacokinetics and bioavailability of salbutamol, Eur. J. Drug Metab. Pharmacokinet. 22 (2) (1997) 145–150.