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Correlation of 'in vitro' release and 'in vivo' absorption characteristics of four salbutamol sulphate formulations

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Abstract

The purpose of this study was to investigate the possibility to develop different levels of correlation between 'in vitro' dissolution parameters and 'in vivo' pharmacokinetic parameters for four salbutamol sulphate formulations: two commercially available formulations (Ventolin® Oral and Volmax®) and two sustained-release formulations (SG7 and SG14) developed in our laboratory. A level A correlation of 'in vitro' release and 'in vivo' absorption could be set up for individual plasma level data by means of the deconvolution method. Linear correlations could be obtained when dose fraction released 'in vitro' was plotted vs. dose fraction absorbed 'in vivo', with correlation coefficients between 0.97 and 0.99 for the formulations studied. A second level of correlation between mean 'in vitro' dissolution time (MDT) and mean 'in vivo' residence time (MRT) was performed with a correlation coefficient of 0.81. Finally, it was also possible to establish a good 'in vitro'-'in vivo' correlation when the mean dissolution time 'in vitro' and T_{max} or C_{max} 'in vivo' were compared, but it appeared impossible to establish any correlation between mean dissolution time (MDT) and AUC.

Keywords: Release, 'in vitro'; Absorption, 'in vivo'; Salbutamol; 'In vitro-in vivo' correlation

1. Introduction

Controlled-release pharmaceutical dosage forms may offer one or more advantages over

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conventional or inmediate-release dosage forms of the same drug, including a reduced dosing frequency, or decreased incidence and/or intensity of adverse effects, a greater selectivity of pharmacologic activity, and a more constant or prolonged therapeutic effect. A fundamental question in evaluating a controlled-release product is whether formal clinical studies of the safety and efficacy of the dosage form are needed or whether a pharmacokinetic evaluation will suffice.

The 'in vitro' dissolution test is important for the purpose of: (a) providing necessary process control, (b) stability determinations of the relevant release characteristics of the product and (c) facilitating certain regulation, determinations and judgments concerning minor formulation changes (Skelley et al., 1990). Correlation between 'in vitro' testing and 'in vivo' performance are encouraged and guidelines were recently published in the proceedings of a controlled release workshop (Blume et al., 1995) and a chapter about 'in vitro' and 'in vivo' evaluation of the dosage forms is included in the last edition of the USP 23 (1995).

The purpose of this study was to investigate the possibility to develop different levels of correlation between 'in vitro' dissolution parameters and 'in vivo' pharmacokinetic parameters for four salbutamol sulphate formulations: two commercially available formulations (Ventolin® Oral and Volmax®) and two sustained-release formulations (SG7 and SG14), developed in our laboratory and previously evaluated from a biopharmaceutical point of view (Bonferoni et al., 1992; Hernández et al., 1994).

2. Material and methods

2.1. Dosage forms

Intravenous administration of salbutamol was made with a commercial solution of salbutamol sulphate (Ventolin® i.v.). Four different formulations of the drug were used for oral administration: a commercial available inmediate-release oral formulation (Ventolin® Oral), an osmotic pump (Volmax®) and two sustained-release formulations developed in our laboratory (SG7 and SG14).

Table 1
Granulation compositions of the matrices

	SG7	SG14
Salbutamol sulphate (mg)	9.6	9.6
Methocel K100M (mg) (hydroxypropylmethylcellulose)	100	70
Blanose 7 HFD (mg) (sodium carboxymethylcellulose)	—	30
Talc (mg)	10	10
PVP (mg)	13	13

2.2. Preparation of tablets

The dosage forms developed in our laboratory were based on a matrix design using hydroxypropylmethylcellulose (SG7) or a mixture of this polymer with anionic sodium carboxymethylcellulose (SG14). The composition of the two matrices is given in Table 1. Drug, polymer or polymer blend and talc were wetted in a mortar with a 20% ethanolic solution of PVP and sieved through a 710-μm sieve. After drying and lubrication with additional 3.6% talc and 0.7% magnesium stearate, compression was performed with a single punch tableting machine (Korsch, Berlin, Germany) equipped with a 8-mm convex punch.

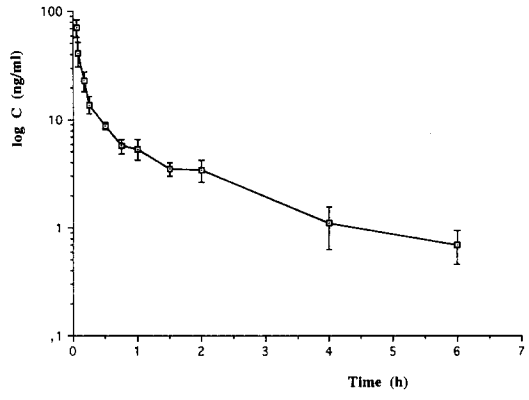

Fig. 1. Evolution of the average plasma levels of salbutamol after its intravenous administration (20 μg/kg) in dogs.

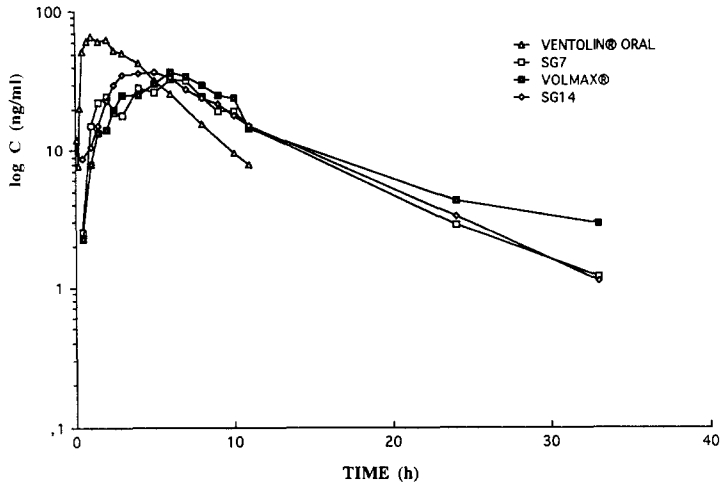

Fig. 2. Evolution of the average plasma levels of salbutamol after oral administration of the four formulations studied.

2.3. 'In vitro' studies

The release test (6 replicates) were performed in water (500 ml, 37°C) using USP 23 apparatus 1 at 100 rpm. One-milliliter samples were drawn at fixed intervals, filtered and assayed by HPLC. The samples of the dissolution assay were evaluated by HPLC according to the method of Mulholland and Waterhouse (1988). HPLC apparatus (Kontron Instruments, Milan, Italy) equiped with a 420-pump and 432 UV-VIS detector was employed. The column was a Hibar Lichrosorb CN (10μm) 250 x 4 mm and the mobile phase a mixture of 0.05 M KH_2PO_4/H_3PO_4 buffer (pH = 3.0) and isopropanol (97:3), run at a flow rate of 1 ml/min. The UV detection was performed at 210 nm wavelength.

Mean dissolution time (MDT) was calculated from the dissolution data with the trapezoidal rule using the program PKCALC (Schumaker, 1986).

2.4. 'In vivo' studies

Five mongrel dogs (weight 24.5 $\pm$ 5.2 Kg) were used in this study. The administration of the dosage forms was carried out following a randomized blocks design. The dosage of the drug in the oral administration was 9.6 mg of salbutamol sulphate and 20 μg/Kg for the intravenous administration. A washout period of 8 days elapsed between the administration of the formulation. Blood samples were drawn at 0.05, 0.08, 0.17, 0.25, 0.75, 1, 1.5, 2, 4 and 6 h for i.v. administration and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, 11, 24 and 33 h after oral administration. Approximately 5 ml of blood were drawn each time from the femoral foreleg vein via a Venflon® catheter in heparinized tubes. The blood samples were centrifuged and the plasma kept frozen (−20°C) until analysis.

Plasma salbutamol was assayed by high performance liquid chromatography (VARIAN 2010), with ion-pair extraction and fluorescence detection (KONTRON SFM 25) according to Hutchings et al. (1983). Briefly, 125 ng of bamethan sulphate (internal standar), 0.2 ml of 0.42 M fosfate buffer (pH = 7.2) and 5 ml of a solution of 0.1 M di-(2-ethylhexyl)-fosfate (DEHP) in chloroform were added to 1 ml of plasma. Once shaken and centrifuged, the organic layer was transferred to a glass tube containing 200 μl of hydrochloric acid. The tubes were agitated and centrifuged. Aliquots of 100 μl of the acid aqueous phase were then injected onto the sample loop. The column was Nucleosil C18 (5μm) 150 x 4.6 mm and the mobile phase (water-acetonitrile, 92:8, adjusted to pH = 2.5 with phosphoric acid) was run at a flow-rate of 1.6 ml/min. The spectrofluorimeter operated at a wavelength of 220 nm (excitation) and 309 (emission). The minimum measurable concentration was 0.5 ng/ml.

Table 2
Pharmacokinetic parameters

	Ventolin ® Oral	Volmax®	SG7	SG14
$t_{1/2}$ (h)	3.0 ± 0.78	7.2 ± 2.1	5.4 ± 1.2	5.8 ± 1.02
AUC (ng h/ml)	386.1 ± 128.4	413.3 ± 95.7	247.0 ± 111.1	406.6 ± 59.1
MRT (h)	5.0 ± 1.7	12.2 ± 4.2	11.7 ± 3.6	10.1 ± 1.1
MIT (h)	3.9 ± 1.3	11.1 ± 4.4	10.5 ± 3.9	8.9 ± 1.5
C_{max} (ng/ml)	68.8 ± 12.2	37.4 ± 6.4	24.7 ± 6.6	42.8 ± 9.7
T_{max} (h)	1.2 ± 0.5	5.8 ± 1.9	6.4 ± 3.0	4.3 ± 1.2

Kinetic analysis of plasma concentration-time data were performed after the different administrations according to non-compartmental approaches from the usual relationships (Gibaldi and Perrier, 1982). The 'in vivo' percent of drug absorbed was calculated by numerical deconvolution using the KINBES® (1991) computer program.

2.5. In vitro-In vivo correlation

Three levels of correlation have been defined according to the USP 23:

2.5.1. Correlation between percent absorbed 'in vivo' versus percent released 'in vitro'

The 'in vitro' dissolution curve of the product is compared with the 'in vivo' absorption curve generated by deconvolution of the plasma levels data. The mean data for the 'in vivo' percent absorbed were plotted versus time and the 'in vitro' drug released versus time were superimposed on the first plot. However, the simplest way to demonstrate a correlation is to plot the fraction absorbed 'in vivo' (obtained by deconvolution) versus the fraction released 'in vitro' at the same time.

2.5.2. Correlation between mean 'in vitro' dissolution time and mean 'in vivo' residence time

In this level of correlation, the mean 'in vitro' dissolution time of the product is compared with either the mean 'in vivo' residence time (MRT) or the mean 'in vivo' absorption time (MIT) of the product derived by using principles of statistical moment analysis.

2.5.3. Correlation between 'in vitro' dissolution parameters and 'in vivo' pharmacokinetic parameters

In this level of correlation, a mean 'in vitro' dissolution time is compared with different pharmacokinetic parameters obtained from 'in vivo' studies. The 'in vivo' pharmacokinetic parameters used for correlations were: the half-life of elimination ($t_{1/2}$), maximum plasma concentration (C_{max}), time to maximum plasma concentration (T_{max}) and the area under the plasma levels curve (AUC).

3. Results and discussion

Figs. 1 and 2 show the evolution of the average plasma levels of salbutamol after the intravenous administration (Ventolin® i.v.) and after the oral administration of the four tested formulations (Ventolin® Oral, Volmax®, SG7 and SG14). The main pharmacokinetic parameters used to establish the 'in vitro'-'in vivo' correlations are listed in Table 2.

The dissolution profiles ('in vitro') were compared with the cumulative absorption profiles ('in vivo') obtained by using deconvolution of the plasma levels for the four formulations studied: Volmax®, SG7, SG14 and Ventolin® Oral (Fig. 3). The 'in vitro' release curves showed less variability than those obtained by deconvolution. The rate of release and of absorption for the formulations Ventolin® Oral and SG7 behaved according to first-order kinetics. With Volmax® and SG14 formulations a zero-order 'in vitro' release and 'in vivo' absorption of salbutamol were obtained during the first 10 h. The 'in vitro' release was faster

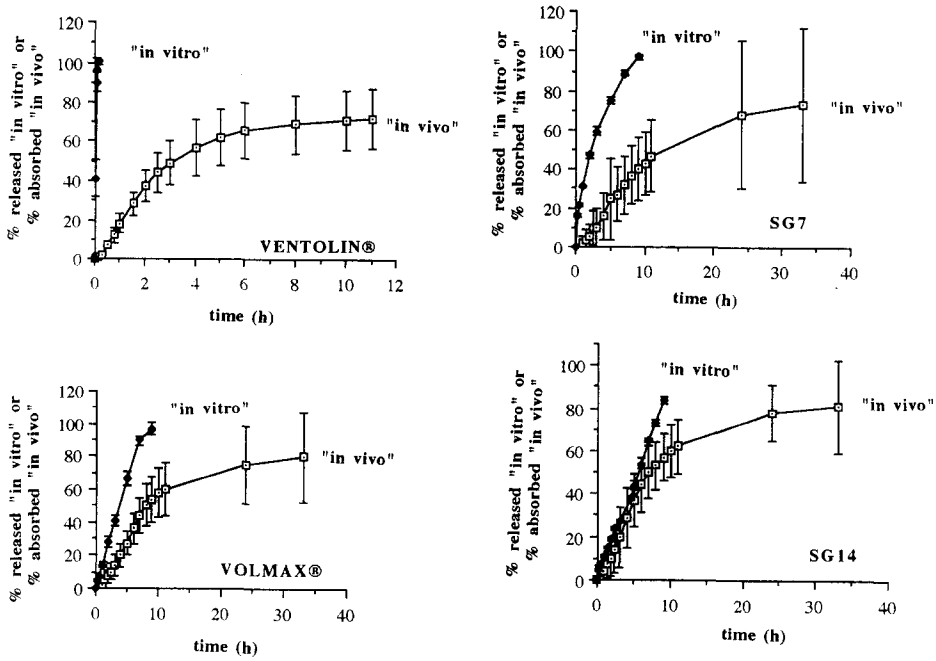

Fig. 3. Comparison of the 'in vitro' release profiles and 'in vivo' cumulative absorption profiles.

than 'in vivo' absorption in all formulations studied, although the most similar 'in vitro' and 'in vivo' kinetics was obtained with SG14. These results are consistent with those obtained by Ferrari et al. (1993) who showed that gastric emptying of the dissolved drug and the dosage form may represent a rate-limitating step in the absorption of salbutamol sulphate released from these dosage forms.

Linear correlation plots for percentage of 'in vivo' dose absorbed and percentage of 'in vitro' dose released are presented in Fig. 4. For the conventional formulation Ventolin® Oral, this correlation could not be stablished. As can be observed, an acceptable correlation was obtained in the case of the three sustained-release formulations allowing a good linear-fitting with correlation coefficients of 0.98, 0.97 and 0.99 for Volmax®, SG7 and SG14, respectively. This kind of correlation is quite important since it represents a point to point relationship between 'in vitro' dissolution and the 'in vivo' input rate of the drug from the dosage form. This is not found with any other correlation and it reflects the complete plasma level curve. Thus an 'in vitro' dissolution curve can serve as surrogate for 'in vivo' performance (USP 23). The slopes of the regression lines were higher than one in all formulations studied. The slopes reported suggest that the 'in vivo' salbutamol absorption rate was slower than the 'in vitro' release rate observed.

Fig. 5 shows the correlation obtained between 'in vitro' mean dissolution time values and 'in vivo' mean absorption time values calculated from the deconvolution curve. It can be observed that as the 'in vitro' mean dissolution time increases, mean absorption time is higher, establishing a linear correlation according to the equation: $MIT_{vivo} = 3.6629 + 2.1015\ MDT_{vitro}$ with a correlation coefficient of 0.82. Similar results were obtained when comparing 'in vitro' mean dissolution time and 'in vivo' mean residence time. When the MDT of the drug from the dosage form is increased, the MRT increases also: $MRT_{vivo} = 4.7818 + 2.1126\ MDT_{vitro}$ with a correlation coefficient of 0.81. These poor correlations could be due to the deviation of linearity of the SG14 formulation as a consequence of the pH-depen-

dent release profile of the sodium carboxymethylcellulose (Baveja et al., 1987).

The correlation between mean dissolution time (MDT) and the selected pharmacokinetic parameters ($t_{1/2}$, T_{max} and C_{max}) is shown in Fig. 6. A poor correlation coefficient was observed between

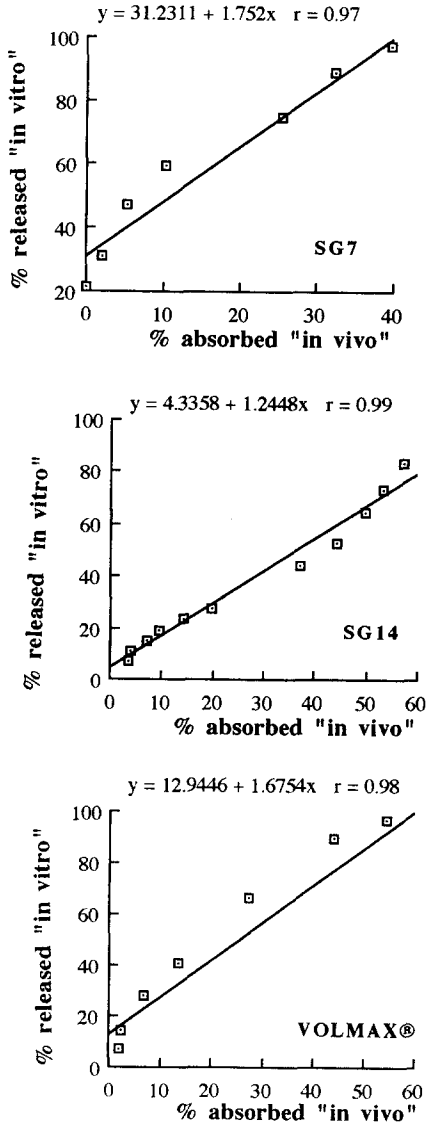

Fig. 4. Plots of mean percentage of dose absorbed versus mean percentage of dose released for the sustained-release formulations of salbutamol sulfate. The line of best fit is shown for each formulation.

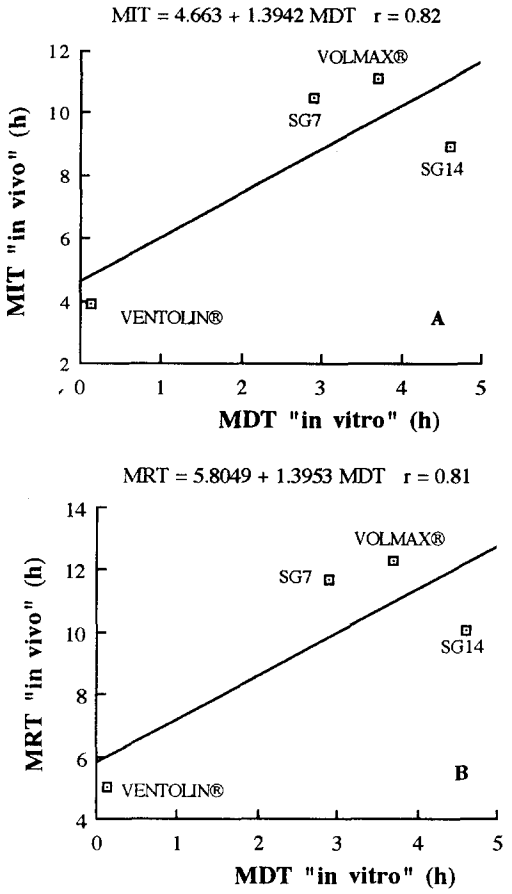

Fig. 5. Correlation between (A) mean absorption time (MIT) or (B) mean residence time (MRT) and 'in vitro' mean dissolution time (MDT).

MDT and $t_{1/2}$, (r = 0.87) for these formulations. However, an excellent quantitative correlation coefficient was observed between MDT and (a) maximum plasma concentration (C_{max}), r = 0.98 or (b) time to maximun plasma concentration (T_{max}), r = 0.98 for salbutamol sulphate formulations. It was impossible to established any confident correlation between MDT and AUC. These results suggest that the MDT obtained by the use of dissolution test is only a good predictor of C_{max} and T_{max} but fails to predict the AUC for these formulations. The last type of correlation represents a single point correlation. It does not reflect the complete shape of the plasma level profile,

which is the critical factor that defines the performance of modified-release products. Since this type of correlation is not predictive of actual 'in vivo' product performace, it is generally only useful as a reference in formulation development or as a production quality control reference procedure (USP 23).

The significant correlation between the 'in vitro' and the 'in vivo' parameters reported here indicates that the 'in vitro' release rate procedure is capable of discriminating between extended-release formulation having different 'in vivo' bioavailabilities. 'In vitro' testing by this procedure certainly represents a viable method to develop modified-release formulations and to monitor production batches for quality assurance. The present in vitro/in vivo correlation methods, which are consistent with Level A, Level B and Level C correlation guidelines described by the FDA/AAPS (Food and Drug Administration/ American Association of Pharmaceutical Scientists) and the USP 23, provide the manufacturer with a valuable 'in vitro' test that can be used to obtain useful information on the 'in vivo' absorption behaviour of such formulations.

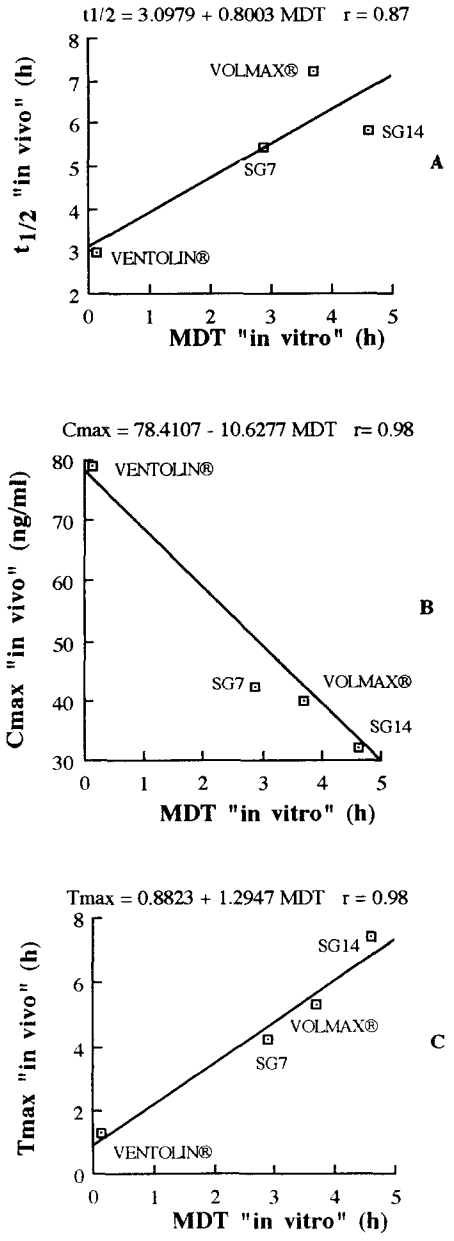

Fig. 6. Correlation between (A) half-life of elimination ($t_{1/2}$) or (B) maximum plasma concentration (C_{max}) or time to maximum plasma concentration (T_{max}) and 'in vitro' mean dissolution time (MDT).

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