In Vitro–in Vivo Correlation of a Modified-Release Oral Form of Ketotifen: In Vitro Dissolution Rate Specification

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Abstract □ The dissolution rate profile of a new modified-release (MR) oral tablet of ketotifen (Zaditen SRO tablet, Sandoz Ltd.) was determined under different conditions (pH, rpm, paddle or basket) with the U.S.P. apparatus. Three different variants of MR tablets were tested. In addition, the in vivo bioavailabilities of these MR tablets were evaluated after a single-dose administration under different conditions (fasting state, with food in morning and/or evening). Several possibilities were evaluated to obtain a correlation between in vitro and in vivo data of the three MR tablets. An excellent linear correlation ($r = 0.997$) was obtained between the cumulative dissolved percent in vitro and the cumulative absorbed percent in vivo at each time under certain conditions. This was obtained in vitro with the dissolution rate performed in distilled water (37 °C) with the U.S.P. apparatus 2 (rotating paddle) and in vivo after a single-dose administration in the morning, fasting state. On the basis of this correlation, of the in vitro dissolution rate for a given variant, and of a simple method of calculation, a reliable prediction of the plasma concentrations obtained following a single dose or at steady state was found. The reliability of this prediction was validated from variants of MR tablet presenting different in vitro dissolution rate profiles and with an upscaled batch which was tested in vivo. This result allows the specifications (upper and lower limits) of the dissolution rate for the MR tablet to be defined and ensures good in vivo characteristics for the different batches of Zaditen SRO tablets during manufacture.

Ketotifen (Zaditen, Sandoz Laboratories, Basle, Switzerland) is a benzocycloheptathiophene derivative used as an orally active anti-allergic compound, mainly for prophylaxis of asthma.[1] As with other asthma prophylactic drugs, ketotifen has a slow apparent onset of action, and mild sedation is observed particularly at the beginning of treatment. The major indication is the long-term prophylaxis of asthma in daily dose of 2 × 1 mg (morning, evening) in adults and children.[2] The marketed forms are solution, syrup, capsules (1 mg), and tablets (1 mg) that are bioequivalent.[3]

A MR tablet was needed with the intention of providing the following advantages: (*1*) better tolerability by allowing the drug to be taken in the evening, when sedation is usually more acceptable than during the day, (2) improved compliance with a once-a-day administration, without loss of bioavailability, and (*3*) avoidance of the high peak plasma concentration of standard forms.

Numerous systems were tested to obtain slow release of the drug that slowed down the rate of absorption but did not slow down the quantity absorbed. One of these principles showed an excellent pharmacokinetic profile that was confirmed by clinical efficacy.[4] This new form of ketotifen is available as Zaditen SRO tablet.

During the development of this new form of ketotifen, the different variants of the MR tablet showed differences in the in vitro dissolution rate (DR) profile. An important issue to ensure the quality and performance of a marketed MR formulation, is the establishment of in vitro dissolution specification for the different batches manufactured.

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To determine the acceptable range for the DR of the definitive variant of MR tablet, a correlation had to be found between the in vitro DR and the in vivo pharmacokinetic parameters of the MR tablet.

According to the literature,[5–10] the main in vitro–in vivo correlations reported for a slow release form of a drug are obtained between parameters such as mean dissolution time, quantity dissolved at one precise time, rate of dissolution, etc. for in vitro parameters, and area under the plasma concentration time curves, peak plasma concentrations, time to peak plasma concentrations, quantity absorbed at a determined time, etc., for in vivo parameters. However, these single-point correlations, do not ensure the reproducibility of the overall in vivo kinetic profile.

Other approaches have been suggested in which the DR curves are described by an equation of order 1,[11] allowing the prediction of the plasma concentrations from a DR curve without any assumption of a mathematical model to describe this DR curve.[12–14] However, only a few examples can be found in the literature of an accurate prediction of the plasma concentrations from the in vitro DR.[11,15,16]

The present paper describes for the Zaditen SRO tablet (*1*) an approach that provides optimal conditions for assessing the dissolution rate and for performing the pharmacokinetic study to be selected to obtain a good in vitro–in vivo correlation; (2) a possibility of predicting plasma concentrations after single-dose or steady-state administration from a DR curve, and (*3*) the possibility of defining the acceptable range of DR for a given variant of MR tablet to ensure good in vivo pharmacokinetic performances.

Experimental Section

Dosage Forms—The reference forms (immediate release) were the marketed capsules, Zaditen, (1 mg of ketotifen; batches 222, 289, and 329; Sandoz Laboratories, France). Four variants of the MR tablet (2 mg of ketotifen), A, B, C, and D, were manufactured at Sandoz Laboratories, Basle, Switzerland. Variants A, B, and C were manufactured at a pilot size and differ in the quantities of retard ingredient (B ≠ A and C), and in the addition (B and C) or not (A) of a water-soluble coating. Variant D had the same composition as variant C, but was manufactured at industrial scale batch size after improvement of the process.

Dissolution Testing—Four different dissolution tests (DR) were used.

DR1—The in vitro dissolution tests were performed with the U.S.P. apparatus 1 (rotating basket). The basket was rotated at 120 rpm in 500 mL of HCl at pH 1 for 2 h, then at pH 6.8 at 37 °C. The dissolved ketotifen was analyzed with an HPLC method using UV detection. For each variant, the mean curve representing the cumulative percent dissolved in vitro (CPD) versus time was calculated from the results of at least three dissolution tests. Each dissolution test was performed on six tablets.

DR2—The in vitro dissolution tests were performed with the U.S.P. apparatus 2 (rotating paddle). The paddle was rotated at 50 rpm in 500 mL of water at 37 °C with a cycle of pH (from 2.4 to 6.8). The other steps were the same as for DR1.

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DR3—The in vitro dissolution tests were performed with the U.S.P. apparatus 2 (rotating paddle). The paddle was rotated at 50 rpm in 500 mL of water at 37 °C. The other steps were the same as for DR1.

DR4—The in vitro dissolution tests were performed as for DR1 but with the USP apparatus 2 (rotating paddle).

Pharmacokinetic Study Design—Young healthy volunteers (58) of both sexes participated in four different studies. All subjects gave their written consent after the study objectives and procedures had been explained to them. All study protocols were submitted and accepted by an Ethics Committee.

Study I—The comparative bioavailability of three variants (A, B, and C) of MR tablets (2 mg) was evaluated and compared with the reference capsules (1 mg). The study was conducted as a four-period cross over trial in 15 subjects after administration of a single dose (2 mg). Administration was in the morning, 2 h before a standardized continental breakfast. The pharmacokinetic evaluation was performed over a 48-h period.

Study II—The influence of food on the bioavailability of the MR tablet was evaluated after administration of a single dose (2 mg) in a three-period crossover trial in 15 subjects. The drug was administered as reference capsules 2 h before dinner, as MR tablet (variant B) 2 h before dinner, and as MR tablet (variant B) 30 min after starting dinner. The pharmacokinetic evaluation was performed over a 48-h period.

Study III—The bioavailability at steady state was evaluated in 12 subjects in a two-period crossover trial. Each subject received a 2-mg daily dose of ketotifen for 8 days. The dosage regimen was reference capsules (1 mg) twice a day (BID) or MR tablet (2 mg) once a day (OAD) (variant A) in the morning. The drug was taken in each case 10 min before food intake. The pharmacokinetic evaluation was performed over a 24-h period on day 8.

Study IV—The bioavailability at steady state was evaluated in 16 subjects in a two-period crossover trial. Each subject received a 2-mg daily dose of ketotifen for 9 days. The dosage regimen was reference capsules (1 mg) BID (8 a.m.–8 p.m.) or MR tablet (2 mg) OAD (variant D) in the evening (8 p.m.). The drug was taken in each case 30 min after starting food intake. The pharmacokinetic evaluation was performed over a 24-h period from day 8 (8 p.m.) to day 9 (8 p.m.).

Assay of Ketotifen in Plasma—Plasma ketotifen concentrations were determined by an RIA.[17] The performances of the RIA were sufficient to follow the plasma kinetics of ketotifen in the four studies. Typical results for the interassay variations of the quality control samples analyzed during the assays of the study samples were 32, 11, 12, and 9% for 40, 250, 400 and 1500 pg/mL concentrations, respectively.

Pharmacokinetic Analysis—Comparison between MR tablets and reference capsules after single-dose administration was made on the following parameters: the maximum plasma concentration (C_{max}), the ratio of C_{max} (RC_{max}), time to the maximum plasma concentration (t_{max}), and relative bioavailability of MR tablet versus reference capsule from the ratio of the area under the plasma concentration time curves ($AUC_{0-\infty}$). The $AUC_{0-\infty}$ was calculated by trapezoidal rule plus extrapolation to infinity with the β value of the terminal half-life (see below).

For the steady-state studies, the comparison was done on the following parameters: C_{max}, RC_{max}, t_{max}, the minimum plasma concentration (C_{min}), ratio of C_{min} (RC_{min}), relative bioavailability of MR tablet versus reference capsule (ratio of AUC for 0–24-h interval) and fluctuating index (FI):

$$FI = \frac{C_{max_{ss}} - C_{min_{ss}}}{C_{av}} \quad (1)$$

In eq 1, C_{av} is defined as follows:

$$C_{av} = \frac{AUC_{ss}\ [0\text{–}24\ h]}{\tau} \quad (2)$$

In eq 2, τ is the dosing interval (24 h). The plasma concentration profiles of ketotifen following a single oral administration indicate two-compartment characteristics.

The individual plasma concentration data obtained after administration of the reference capsule were fitted using Pharm program[18] with the following general equations:

$$C(t) = -Ce^{-ka(t-\Delta t)} + Ae^{-\alpha(t-\Delta t)} + Be^{-\beta(t-\Delta t)} \quad (3)$$

In eq 3, C = A + B, $C(t)$ is the plasma concentration at time "t" [$C(t) = 0$ when $t \leq \Delta t$], t is the time after dosing, Δt is the lag time, A, B, and C are pre-exponential factors, and ka, α, and β are rate constants of the plasma concentration–time curve. The microconstants k_{21}, k_{el}, and k_{12} were then calculated.[19]

In Vivo Fraction Absorbed—The Loo–Riegelman method[19] for a two-compartment model was used to evaluate the cumulative percent absorbed in vivo (CPA) at each time. In the absence of iv administration, the microconstants obtained after the oral administration of the reference form were used. In each case, the CPA for the reference capsule was assumed to be 100% at infinity. The individual CPA at infinity for the MR tablet was calculated from the relative bioavailability of the MR tablet versus the reference capsule. The calculation was done for each subject and each administered variant of study I and also for the mean plasma concentration–time curves of studies I and II for each variant.

In Vivo–in Vitro Correlation—Two kinds of correlation were evaluated. First, for the same value of CPA and CPD between the two corresponding in vivo and in vitro times, and second, for a given time, between the mean CPA in vivo and the mean CPD in vitro.

Predicted Plasma Concentration after Single-Dose Administration from the in Vitro DR—The Loo–Riegelman method allows the calculation of the input kinetics from the plasma concentration. It is possible with the Loo–Riegelman equations to predict, from in vitro DR, the plasma concentration of MR formulation. The calculation is based on in vitro DR data for the variant of MR tablet concerned, the in vitro–in vivo correlation found, and the mean $AUC_{0-\infty}$ of the reference capsule for the study concerned.

The first step in the calculation consists of calculating the fractions dissolved–absorbed in vivo at each time on the basis of the in vitro DR values and of the correlation. The second step allows the plasma concentrations to be calculated at each time for the MR tablets, based on the microconstants, on the $AUC_{0-\infty}$ of the reference, and on the fraction dissolved–absorbed in vivo. Details of these calculations are given in the *Appendix*. Plasma concentrations are thus calculated for the 0–24 h interval after a single-dose administration.

Predicted Plasma Concentration at Steady State—To compare the plasma kinetics that could be expected for the MR tablet with a once daily administration of 2 mg and for the reference capsule with a twice daily administration of 1 mg, steady-state plasma concentration profiles were simulated. Because ketotifen presents a linear kinetics (personal data), curves were obtained by the superposition principle, using linear interpolated results of the plasma concentration following single-dose administration and extrapolation to infinite time using the mean β-phase ($\beta = 0.039\ h^{-1}$) of the reference capsule (study I).

For the reference capsule, the plasma concentrations following single-dose administration were the experimental mean values obtained during the 0–24-h interval following administration of the reference capsule in study I. For the MR tablet, the plasma concentrations following single-dose administration were the predicted plasma concentrations obtained from the in vitro DR. The simulation was carried out over 7 days, which was long enough to obtain the steady-state profile for ketotifen.

To take into account the differences in AUC observed for the reference in the different studies, a weighting factor was introduced. This difference in AUC for the reference is the consequence of the inter-population variations in clearance and also of the inter assay study variations. The weighting factor used (f) was as follows:

$$f = \frac{AUC_{ss}\ [0\text{–}24\ h] \text{ of reference capsule study III or IV}}{AUC_{0-\infty} \text{ of reference capsule study I}} \quad (4)$$

This weighting factor was applied to the reference capsule and to the MR tablet for the study under consideration.

Results and Discussion

In Vivo Absorption—It was interesting to compare for a determined variant the CPA calculated from the mean plasma concentrations and from the mean CPA obtained from individual plasma concentrations. These comparisons were performed for the three variants tested during study I, whatever the variant considered. A good concordance was observed. An example of this good result is shown for variant A in Figure 1. These results allow us to conclude that it is possible to calculate the CPA directly from the mean plasma concentration–time curve. This allows the length of the calculation to be reduced and, with the mean plasma concentration–time curve, the absorption kinetics to be evaluated.

The influence on the CPA profiles of the conditions of administration was then tested. The CPA profiles of ketotifen

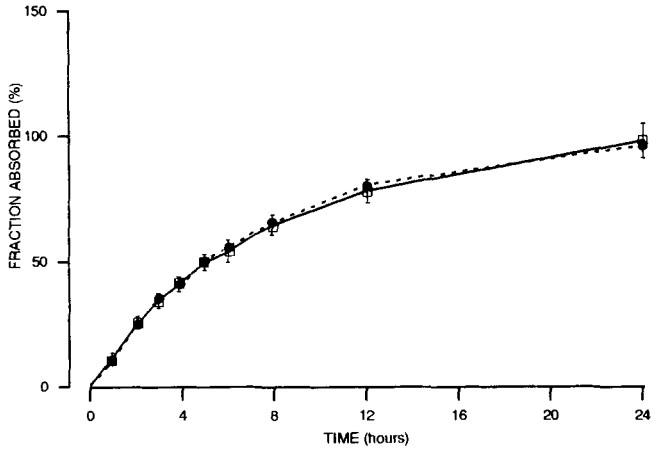

Figure 1—Cumulative percent absorbed versus time from variant A (Study I). Key: (—□—) mean ± SEM of the individual (n = 15) cumulative percent absorbed; (- -●- -) cumulative percent absorbed deduced from the mean plasma concentrations.

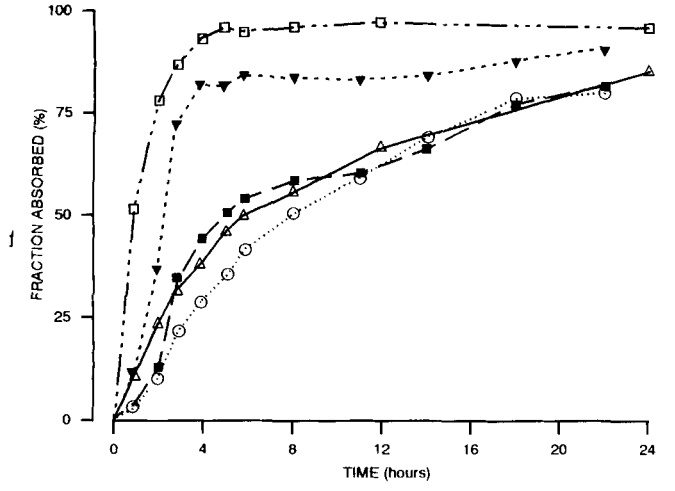

Figure 2—Mean cumulative percent absorbed versus time. Key: (—□- -) reference study I, 2 h before breakfast; (- -▲- -) reference study II, 2 h before dinner; (—△—) variant B, study I, 2 h before breakfast; (—■—) variant B, study II, 2 h before dinner; (···○···) variant B, study II, during dinner.

after administration of the reference capsule in studies I and II 2 h before breakfast and 2 h before dinner are shown in Figure 2. A clear slowing down of the absorption phase is obtained in the evening compared with the morning. This has been observed for other drugs by different authors[10,20-24] and also for other drugs in our laboratory. This slowing down is likely due to the difference in the state of the gastrointestinal system after a night of fasting or after a day with breakfast and lunch and may be due to variations in blood flow.[25] The activity of the subjects seems less relevant as the subjects had a similar activity in the morning and in the evening (hospital room).

For the MR tablet, the CPA profiles of ketotifen after administration of variant B were evaluated in studies I and II, 2 h before breakfast, 2 h before dinner, and during dinner. As for the reference, a slowing down of absorption is also observed in the evening compared with the morning (Figure 2). Moreover, drug intake during the dinner also reduces the rate of the absorption phase in comparison with before the dinner; this has been observed for other drugs.[26,27] However, in all these conditions, no appreciable difference in the quantity absorbed of the reference capsules or of the MR tablets was observed.

With the aim of standardization and taking into account the different tests, the absorption in the morning, after a night of

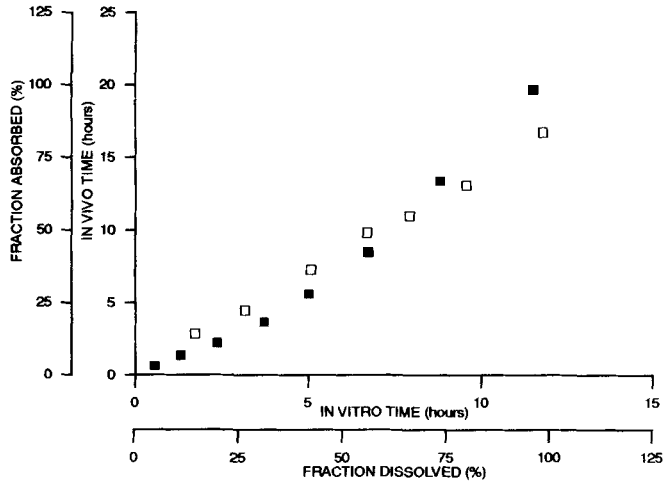

Figure 3—Correlation for variant B between (□) the cumulative percent dissolved and the cumulative percent absorbed for a same time (r = 0.997) and (■) the time in vivo and the time in vitro for a same percent absorbed/dissolved (r = 0.985).

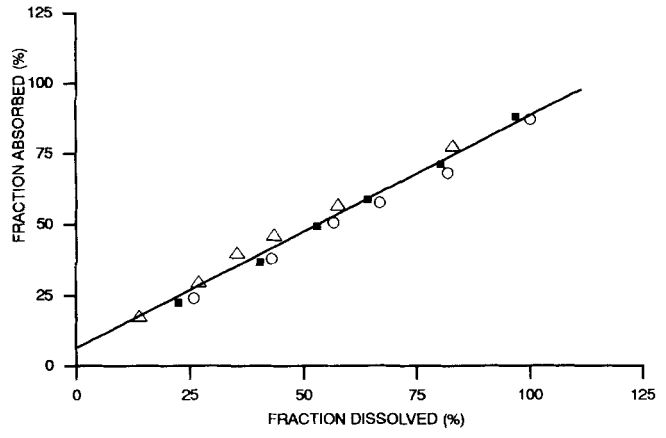

Figure 4—Linear correlation between the percent dissolved for DR3 conditions and the percent absorbed from study I, for variants (■) A, (○) B, and (△) C.

fasting, and 2 h before breakfast, was chosen to correlate the in vitro and the in vivo data.

In Vitro and in Vivo Correlation—An example of the two types of the in vivo–in vitro correlations tested are shown in Figure 3 for variant B. The best linear correlation is observed between the CPA and CPD in a given time. The same results are true for variants A and C. For all the four DR tested and for each variant, good in vivo–in vitro correlations are obtained; however, the best result is obtained with the DR3 conditions. For example, for variant B, the coefficients of correlation were 0.994, 0.990, 0.997, and 0.989 for DR1, DR2, DR3, and DR4 respectively.

Taking into account all these results, the in vivo–in vitro correlation finally chosen is that obtained with the in vitro data in DR3 conditions and in vivo data from study I. The data of variants A, B, and C were put together to obtain the more representative correlation for the MR tablets. The Passing and Bablock method[28] was used to calculate the line of correlation between the in vitro data and the in vivo data (Figure 4).

Single-Dose Expected Plasma Concentrations from in Vitro DR—By the in vitro DR and the method described in the *Appendix*, plasma ketotifen concentrations were calculated for MR tablet variants A, B, and C after single-dose administration. This back calculation was done to verify the reliability of this approach. When the calculated plasma concentrations are

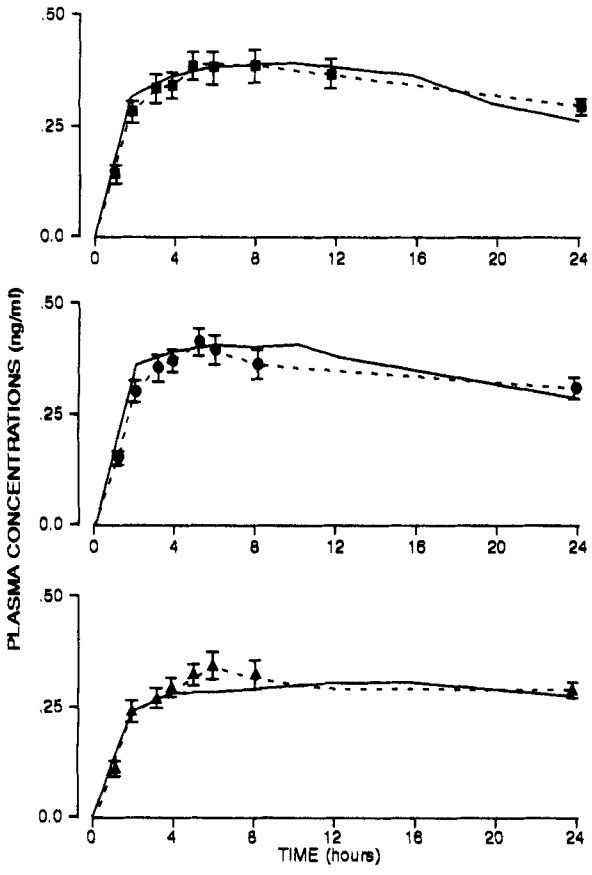

Figure 5—Measured plasma ketotifen concentration versus time curves (- - - -) after a single oral administration of 2 mg (mean ± SEM; n = 15). Key: individual values of variants (■) A, (●) B, and (▲) C from study I; (—) predicted values from in vitro dissolution rate.

compared with those measured in study I (Figure 5), a good agreement is observed between the two curves for each variant.

Steady-State Expected Plasma Concentrations from in Vitro DR—The results comparing the simulated plasma concentrations with those measured in the steady-state studies for the reference and the MR tablets are shown in Figure 6 for study III and study IV. For the reference capsule, a discrepancy is observed between the simulated data and the experimental data. This is because the nycthemeral effect observed for the experimental data is not taken into account by the simulation. Moreover, a larger difference between the simulated and experimental data is observed for study IV than for study III. In fact, the simulated values are deduced from an administration in fasting state 2 h before breakfast, and during study IV the drug was taken during breakfast and during dinner. On the other hand, in study III the drug was taken 10 min before food intake. However, other pharmacokinetic studies performed with ketotifen had demonstrated that the nycthemeral effect has an effect on the rate of the absorption phase between morning and evening administrations, but not on the quantity of drug absorbed (personal data).

For the MR tablet, a good concordance is observed between the simulated and the experimental data of study III. The slight difference during the first part of the kinetics could be explained by the difference in regard of food intake. For study IV the discrepancy is due to the same reason as for the reference. Nevertheless, it is important to compare the conclusions deduced from the simulation and from the experimental data of the two steady-state studies. In Table 1, the main pharmacokinetic parameters of the MR tablets given OAD versus reference capsules given BID are summarized for both cases. For all the tested parameters, quite a good agreement is observed between the simulated data and the experimental data from the two steady-state studies and the two variants, A and D. The results deduced from the simulation have a tendency to put the MR tablets pharmacokinetic performances at a disadvantage compared with those of the reference capsules. We thus felt justified in using the simulated data to evaluate different DR of this MR tablet variant in comparison with the reference capsule.

In Vitro–in Vivo Limits—Based on the in vitro DR of different variants of MR tablet and on the results observed in vivo, upper and lower limits of in vitro DR were proposed for release specifications of a batch of Zaditen SRO tablets (Table 2). The in vivo performances of the variant given OAD corresponding to these upper and lower limits of dissolution tests were compared on the basis of simulation with the reference capsules given twice a day (Table 3). From these parameters, the theoretical variants corresponding to the upper and lower limits of dissolution rate ensure good performances for the MR tablets given OAD.

The only parameter that could be debatable is the bioavailability corresponding to the lower limit with a value of 78%, which is outside the ±20% rule. However, in Table 1, one can see that the bioavailability was higher in the experimental results than in the simulated results for variant D (+10%) and close to the upper limit. This value of 78% calculated from the simulation is thus underestimated, and the lower-limit DR could be accepted.

From these results, it can be concluded that the upper and lower limits of in vitro DR defined as specifications for a MR tablet of ketotifen ensure that the different batches of ketotifen that comply with this criteria will perform well in vivo.

Conclusions

The specifications of the in vitro dissolution tests for a pharmaceutical form of a drug intended for oral administration are not always easy. However, these specifications are necessary to ensure that the bioavailability characteristics of the drug administered in this form are reproducible in vivo from batch to batch. This is the main conclusion of the workshop on in vitro–in vivo correlation for MR forms.[29]

For the Zaditen SRO tablet, it was possible to define the best in vitro DR corresponding to the best in vivo condition of administration to obtain the best in vivo–in vitro correlation. This correlation means that the plasma concentrations can be accurately predicted and justifies the lower and upper limits of the in vitro DR for this form. However, one must keep in mind that it is not always possible to test different variants, different conditions of administration by the oral route, and different conditions of in vitro DR for a given form of drug to obtain such a correlation.

Appendix

Details for the Calculation of the Predicted Plasma Concentration (Single-Dose Administration) from the in Vitro DR Data of the MR Form—from the linear correlation between the in vivo fraction absorbed (FA) and the in vitro fraction dissolved (DR) at each time (T).

$$(FA)_T = u(DR)_T + v \qquad (A1)$$

From the Loo–Riegelman method[19]

$$\frac{(X_A)_T}{V_c} = (FA)_T\, k_{el}\, AUC \qquad (A2)$$

$$\frac{(X_A)_T}{V_c} = C_T + k_{el}AUC_T + \frac{(X_P)_T}{V_c} \qquad (A3)$$

In eq A3:

$$k_{el}AUC_T = k_{el}AUC_{T-1} + k_{el}\frac{\Delta t}{2}C_T + k_{el}\frac{\Delta t}{2}C_{T-1} \qquad (A4)$$

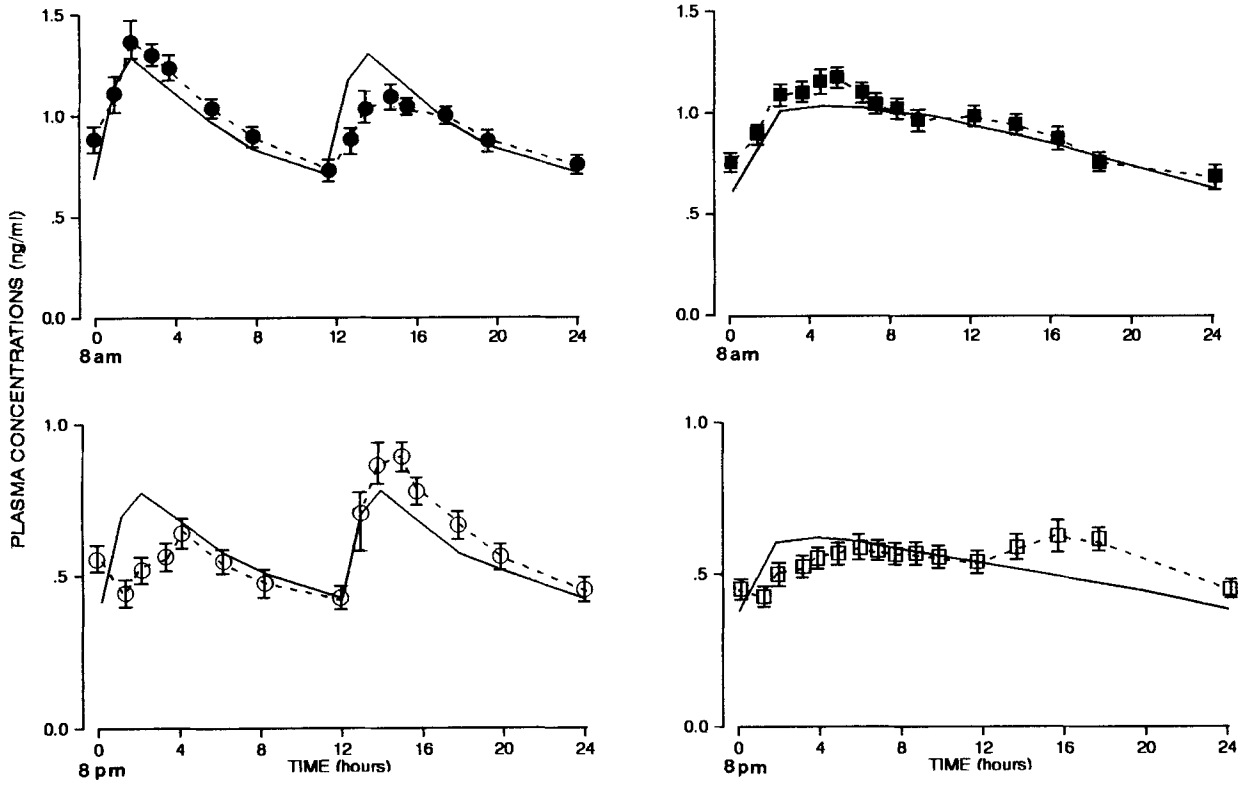

Figure 6—Plasma ketotifen concentration versus time curves at steady state for the reference capsule (1 mg, BID) and MR tablet (2 mg OAD). Key: (- - - -) study III values (mean ± SEM, n = 12) for (●) reference and (■) variant A; (- - - -) study IV values (mean ± SEM, n = 16) for (○) reference and (□) variant D; (—) predicted values from single-dose administration for the reference and from in vitro DR for variants A and D.

Table 1—Relative Pharmacokinetic Parameters at Steady State of MR Tablet (2 mg, OAD) versus Reference Capsule (1 mg, BID): Comparison of Experimental Data (Study III and Study IV) and Predicted Data

Parameter[a]	MR Variant A			MR Variant D		
	Study III (n = 12)	Predicted Data	Difference, %[b]	Study IV (n = 16)	Predicted Data	Difference, %[b]
C_{max} MR tablet/C_{max} Ref.	0.835	0.792	-5.1	0.692	0.761	10.0
C_{min} MR tablet/C_{min} Ref.	0.914	0.859	-6.0	0.978	0.854	-12.7
Bioavailability on AUC 0–24 h, %	92.7	88.6	-4.4	94.1	84.9	-9.8
Fluctuating index[c]						
reference	0.68	0.61	—[d]	0.79	0.61	—
MR tablet	0.55	0.49	—	0.36	0.47	—

[a] Over a 24-h period. [b] Difference (%): [(predicted data – experimental data)/experimental data] • 100. [c] From the mean parameter. [d] —, Not applicable.

Table 2—Release Specifications in DR3 Conditions for the MR Tablet of Ketotifen

Time, h	Dose Dissolved, %	
	Lower Limit	Upper Limit
4	20	50
8	40	75
24	80	100

$$\frac{(X_P)_T}{V_c} = \frac{(X_P)_{T-1}}{V_c} e^{-k_{21}\Delta t} + \frac{k_{12}C_{T-1}}{k_{21}}(1 - e^{-k_{21}\Delta t}) + \frac{k_{12}}{2}\Delta t(C_T - C_{T-1}) \quad \text{(A5)}$$

In eqs A2–A5, $(X_A)_T$ is the total amount of drug absorbed at time T, V_c is the apparent volume of the central compartment, C_T is the concentration for MR form at time T, AUC_T is the AUC of the MR form from time 0 to time T, $(X_P)_T$ is the amount of drug in the peripheral compartment at time T, Δt is the difference between T and $T - 1$, k_{el}, k_{21}, and k_{12} are the microconstants obtained from the reference form in study I, and AUC is the AUC value (0 to ∞) for the reference form.

Table 3—Predicted Pharmacokinetic Parameters of Ketotifen at Steady State for the Upper and Lower Limits Dissolution Rate Specification for the MR Tablet in Comparison with the Reference Capsule

Parameter	Reference Capsule (1 mg BID)	MR Tablet (2 mg OAD)	
		Lower limit	Upper limit
Bioavailability	100%[a]	78%	93%
Ratio C_{max}[b]	—	0.69	0.87
Ratio C_{min}[b]	—	0.88	0.91
Fluctuating index	0.61	0.36	0.54

[a] By definition. [b] MR tablet versus reference capsule.

From eqs A2–A5, predicted plasma concentrations at each time T are as follows:

$$C_T = \frac{(X_A)_T}{V_c} - \frac{(X_p)_{T-1}}{V_c} e^{-k_{21}\Delta t} + C_{T-1} k_{12} \frac{\Delta t}{2} - C_{T-1} \frac{k_{12}}{k_{21}} 1 - e^{-k_{21}\Delta t} - C_{T-1} k_{el} \frac{\Delta t}{2} - \mathrm{AUC}_{T-1} k_{el} / 1 + k_{12} \frac{\Delta t}{2} + k_{el} \frac{\Delta t}{2} \quad \text{(A6)}$$

The concentrations were calculated step by step (Δt = 0.5 h) to obtain the predicted plasma concentration–time curve after a single oral dose of the MR tablet considered.

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