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**Title:** A question based approach to drug development  
**Issue Date:** 2003-09-10
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Funded by: Institut de Recherches Internationales Servier (i.r.i.s.)

Comparison of an oral solution and an oral sustained release formulation of rilmenidine in eight healthy volunteers and correlation with *in vitro* sustained release properties
Abstract

**Rationale**  Rilmenidine is a centrally acting antihypertensive. At the present time, the dosage for rilmenidine is 1 mg once a day, which in some patients needs to be increased to 1 mg twice a day. In order to increase the duration of the effect without increasing the occurrence of peak-dose related side effects, a sustained release (sr) formulation has been developed at a dose of 2 mg. This study aimed to investigate the relationship between *in vitro* and *in vivo* characteristics of dissolution of the slow release formulation. Secondly, the clinical effects and pharmacokinetics of this formulation of rilmenidine compared to a solution in healthy volunteers were investigated.

**Methods**  This was a double-dummy, double blind, randomised, two-way cross-over study in four healthy male and four healthy female volunteers with a six days washout between administrations. Rilmenidine was administered either as a 1 mg solution or a 2 mg sr tablet. Blood samples were taken prior to dosing and at various times up to 36 hours after administration and plasma analysed for unchanged rilmenidine. Deconvolution was used to determine the *in vivo* dissolution of the tablet, which was compared to the *in vitro* dissolution using linear regression. In order to estimate the prediction error of this correlation, the observed *in vivo* results were compared with the predicted *in vivo* kinetics according to the appropriate Food and Drug Administration (FDA) guideline. The clinical effects were evaluated by blood pressure, heart rate and visual analogue scales (VAS) of alertness, mood and calmness.

**Results**  The slope of the mean *in vitro-in vivo* dissolution correlation was 1.1 with a range from 0.71 to 1.7. The average predicted area under the curve (AUC) and maximum observed concentrations (Cmax) deviated 6.7% and 12% from the observed values. The mean absolute average internal prediction errors of the *in vitro-in vivo* correlation (IVIVC) were 32% for AUC and 14% for Cmax. Cmax values were 3.7 ± 0.77 ng·ml⁻¹ with the solution, and 2.6 ± 0.32 ng·ml⁻¹ after the tablet, normalised to a 1 mg dose. These concentrations were reached later for the sr formulation than for the solution (5.4 ± 0.52 h compared with 2.1 ± 0.79 h). The time during which the concentration was greater than 75% of Cmax (t75) was 3.4 h longer for the tablet than for the solution (95% confidence interval: 0.5, 6.3 h). The relative bioavailability of the tablet compared to the solution was 126 ± 54% (coefficient of variation 43%). Both preparations showed similar treatment effects on blood pressure and alertness VAS, with a significantly earlier maximum for the solution (around 3½ hrs) than for the slow release tablet (about 5-6 hours).
**CONCLUSION** Although the internal prediction errors of the *in vitro*- *in vivo* correlation exceeded FDA guideline values, the *in vitro* dissolution kinetics are predictive of the *in vivo* dissolution kinetics. However, the pharmacokinetic properties of rilmenidine appear to be highly variable as illustrated by the high variability in relative bioavailability. The clinical effects of the rilmenidine 2 mg tablet and the 1 mg solution were not statistically significantly different.

**Introduction**

Rilmenidine (2-(dicyclopropylmethyl)-amino-2-oxazoline) is registered as an anti-hypertensive drug in several European countries. Rilmenidine is a centrally acting drug with binding selectivity to I₁ imidazoline receptors over α₂-adrenoceptors. Early clinical studies have indicated that after single administration the drug has a dose-dependent blood pressure lowering effect at doses of 0.5 mg or higher. The maximal effect occurs between 2-3 hrs after drug administration and lasts a minimum of 12 hours. Comparative studies in hypertensive patients have shown that the drug effectively lowers blood pressure compared to congeners like clonidine at equipotent doses. In the same dose range, mild sedation and reduced salivary flow have been reported, although these side effects are considerably less than for the nonspecific α₂ agonists. The drug is commonly prescribed in a dose of 1 mg orally once daily, but some patients require twice daily dosing. In order to increase the duration of the effect without increasing the occurrence of peak-dose related side effects, a 2 mg tablet has been developed which has sustained release (SR) properties *in vitro*. This formulation is intended to provide around-the-clock therapeutic drug concentrations after a once daily administration. To aid in the optimisation of the sustained release profile of novel formulations, the dissolution characteristics were compared *in vitro* and *in vivo* for a 2 mg tablet. Deconvolution techniques were used to determine the *in vivo* sustained release dissolution profile, and a 1 mg solution was used as an ‘immediate release’ form to correct for the absorption of rilmenidine. The SR dissolution profile was subsequently compared to the *in vitro* characteristics of the new tablet. This study aimed to investigate the relationship between *in vitro* and *in vivo* characteristics of dissolution of the slow release formulation. Secondly, the clinical acceptability and pharmacokinetics of this formulation of rilmenidine compared to a solution in healthy volunteers were investigated.
Methods

Study design

This was a double-dummy, double blind, randomised, two-way cross-over study in eight healthy volunteers with a washout between administration of at least six days.

Subjects

Subjects were male or female subjects, healthy as determined during screening, who gave signed informed consent. The study was approved by the Medical Ethics Review Board of Leiden University Medical Center, and performed according to the principles of the Helsinki Declaration. Eight (4 males, 4 females) subjects completed the study.

Drug administration

All subjects received a sustained release formulation of rilmenidine, 2 mg (active treatment) with the solution vehicle as placebo or a rilmenidine solution, 1 mg (active treatment) with sustained release placebo tablet. The sustained release formulation and placebo tablets were produced by Servier, Gidy France. Servier also produced a rilmenidine 1 mg/ml solution, according to GMP procedures. One ml of this solution was further diluted by adding 150 ml water. This final dilution was prepared the afternoon before dispensing and placed in a refrigerator (4°C) overnight.

Sampling

Subjects were studied after an overnight fast (with the exception of occasional water). Alcohol and xanthine containing food and beverages were not allowed from 12 hours before until 36 hours after dosing. A cannula was inserted in a forearm vein to facilitate repeated blood sampling. Samples were collected pre-dose and ½, 1, 1½, 2, 2½, 3, 4, 5, 6, 8, 10, 12, 14, 18, 24, and 36 hours after drug administration in heparin-containing polypropylene tubes (Sarstedt ®) for rilmenidine assay. The tubes were immediately centrifuged for 10 minutes at 4°C and 1500 g and plasma was subsequently divided into two aliquots, frozen and stored at -20°C until analysis. Four
hours after drug administration a standardised lunch was provided and a
dinner was given after ten hours. Subjects went home after 24 hours and
returned to the research unit 36 hours after drug administration for final
measurements. The same procedure was repeated in the second study
period.

**Drug concentration analysis**

Rilmenidine concentrations were determined using gas chromatography-
mass spectrometry (gc-ms) following liquid-liquid extraction according to
the method described by Ung et al.

**In vitro dissolution**

SR tablets containing 2 mg rilmenidine were placed into a 37 °C medium
of 0.05 M phosphate buffer at pH 6.8 (according to the FDA guideline) using
USP apparatus II (paddles). Samples (10 ml) were taken at 0, 1, 2, 4, 8, 12 and
16 hours. After filtration of the samples through a 10 µm polypropylene filter,
an aliquot (5 µl) was injected onto the hplc column (Nucleosil 100-3 C 18
(Macherey Nagel), 150 x 4.6 mm). The concentration of rilmenidine was
determined spectrophotometrically at 205 nm by reference to a calibration
curve.

**Pharmacokinetic analysis**

A non-compartmental pharmacokinetic analysis was performed for each
subject and each treatment. The estimated parameters were the maximum
observed concentration (C<sub>max</sub>; normalised to a 1 mg dose assuming linear
kinetics, C<sub>max,norm</sub>) and corresponding t<sub>max</sub> as well as the last measurable
concentration (C<sub>last</sub>). The area under the concentration versus time curve
from 0 to C<sub>last</sub> was calculated using the linear trapezoidal rule for rising or
static concentrations and the logarithmic trapezoidal rule for declining levels
(AUC<sub>t</sub>; normalised to a 1 mg dose assuming linear kinetics, AUC<sub>t,norm</sub>).
The terminal half life (t<sub>A</sub>) was estimated using the slope of the elimination
phase. The concentration 24 hours after dosing was also determined (C<sub>24</sub>).
Additionally, the time interval between administration and first measurable
concentrations (t<sub>lag</sub>) and the time during which the concentration was
equal to or greater than 75% of the C<sub>max</sub> (t<sub>75</sub>) were estimated. The relative
bioavailability of the sr tablet compared to the solution was estimated according to the following equation:

\[ F_{rel} = 100 \cdot \frac{D_{sol} \cdot AUC_{tab}}{D_{tab} \cdot AUC_{sol}} \]

were \( D_{sol} \) and \( D_{tab} \) are the doses for the solution and tablet, and \( AUC_{sol} \) and \( AUC_{tab} \) are the \( AUC_t \) values for the solution and the tablet respectively.

Compartmental modelling was carried out for the solution data of each subject using WinNonlin software version 3.1 (Pharsight Corp, Mountain View, CA) in order to provide parameters for the numerical deconvolution. A mono- or bi-exponential model, with or without lag time was fitted to the data and the best model fit assessed by comparison of the value of the Akaike Information Criterion (AIC). Coefficients and exponentials from the model fit with the lowest value for AIC were used for the subsequent deconvolution analysis.

### Deconvolution analysis

Numerical deconvolution was performed using pcdcon software version 3.0 (William R. Gillespie, Ph.D., The University of Texas at Austin) according to the method described by Gillespie et al. The model-fitted coefficients and exponentials for the solution were used to describe the unit impulse response. The input of rilmenidine was deconvolved from these two profiles to provide a percentage cumulative amount dissolved (equivalent to the \textit{in vivo} dissolution). The \textit{in vivo} dissolution profile for each individual subject was related to the mean \textit{in vitro} dissolution profile for the sr tablet using linear regression. In addition, an average \textit{in vivo} dissolution profile from all subjects was related to the \textit{in vitro} dissolution profile, yielding a predicted \textit{in vivo} dissolution profile.

In order to estimate the predictive value of this \textit{in vivo}-\textit{in vitro} correlation, internal prediction errors were assessed according to the appropriate FDA guideline. The predicted \textit{in vivo} dissolution of the tablet was convolved with the individual solution concentration profiles. This resulted in predicted \( AUC \) and \( C_{max} \) values for the tablet that were compared to actually observed values, and absolute percent prediction errors were calculated for both individual and mean group values.
Pharmacodynamic determinations

Blood pressure and heart rate were measured immediately before drug sampling (except at 18 hours), and at ¼ and ¾ hours after the drug administration. These vital signs were measured after the subject had been sitting in a semi-recumbent position for at least 5 minutes. An automated blood pressure monitor (MPV1072, Nihon Kohden, Japan) was used, which displays an average value for two duplicate measurements at each time point. Visual analogue lines as originally described by Norris were also used in this study. The subjects were asked to indicate with vertical marks on 16 horizontal 100-mm lines how he/she felt at that moment. The 16 categories were (Dutch translations of): Alert/Drowsy, Calm/Excited, Strong/Feeble, Confused/Clear-headed, Well-coordinated/Clumsy, Lethargic/Energetic, Contented/Discontented, Troubled/Tranquil, Mentally slow/Quick-witted, Tense/Relaxed, Attentive/Dreamy, Incompetent/Proficient, Happy/Sad, Antagonistic/Amicable, Interested/Bored and Withdrawn/Gregarious. From this set of lines three factors were derived as identified by Bond and Lader, corresponding to alertness, mood and calmness. These factors were used to quantify subjective central nervous system effects. Visual analogue scores were recorded at ½, 1, 2, 4, 6, 8, 10, 12, 14, 24 and 36 hours. Reports of adverse events were elicited by the question “How do you feel” and by recording spontaneous reports.

Statistical analysis

Pharmacodynamic measurements were characterised by calculating the time of maximum effect ($t_{\text{max}}$) and the corresponding measurement ($E_{\text{max}}$), and the area under the curve (AUEC) over the 0-12 hours time period. These AUECs were subsequently divided by the corresponding time span resulting in a weighted average value. Measures were compared between treatments using paired t-tests. Calculations were performed using SPSS for Windows V10.0.7 (SPSS, Inc., Chicago, IL).

Results

Subject demographics

All subjects completed both study occasions. No serious adverse events occurred during the study. Subjects were 23 years of age (range 18-27 years),
with an average weight of 69.8 kg (range 49.1-87.7 kg) and height of 177 cm (range 161-191 cm). Average pre-dose blood pressures were (systolic/diastolic) 114/62 mmHg (range 97-139/52-72 mmHg) and a heart rate of 65 bpm (range 48-85 bpm).

### Pharmacokinetic parameters

The main pharmacokinetic parameters are represented in Table 1. The mean plasma concentrations after both solution and sr tablet are represented in Figure 1.

T_max was reached on average 3.3h later for the sr formulation than for the solution (95% ci: 2.5, 4.0 h). C_max,norm for the sr tablet was on average 1.1 ng/ml lower for the tablet than for the solution (95% ci: 0.6, 1.6 ng/ml). The time during which the concentration was greater than 75% of C_max (t_{75}) was 3.4 h longer for the tablet than for the solution (95% ci: 0.5, 6.3 h).

Average normalised AUC was similar for both treatments but more variable for the solution than for the tablet (cv of 37% and 17% respectively).

The relative bioavailability of the sr tablet compared to the solution (F_{rel}) was 126 ± 54 % (Mean ± standard deviation). T_{1/2} was similar for both treatments but more variable for the solution than for the tablet (cv of 68% and 43% respectively).

<table>
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<tr>
<th>Parameter</th>
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<th>sr Tablet</th>
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<tr>
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<td>0.77</td>
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**Table 1**

Average pharmacokinetic parameters after oral administration of a 1 mg rilmenidine solution and a 2 mg sustained release tablet including P-values for the difference
**Figure 1**
Average rilmenidine plasma concentration after oral administration of a 1 mg solution (●; solid line) and a 2 mg sustained release tablet (▲; dashed line)

**Figure 2**
Relationship between *in vitro* and *in vivo* dissolution of the 2 mg sustained release tablet (●; solid line). $y = 1.126x + 0.692$, $R^2 = 0.99$, $cv = 44\%$. Dashed line is the line of identity
**In vitro – in vivo correlation of the dissolution**

The relationship between the *in vitro* and mean *in vivo* dissolution is given in Figure 2. The slope of the mean *in vitro – in vivo* correlation was 1.12. The variability in the *in vivo* dissolution was considerable with a range of the individual slopes of 0.71 to 1.67.

The overall difference between the average predicted values and observed values was 6.8% for the *AUC* and 11.9% for the *Cmax*. However, the means of the individual absolute percent prediction errors (the internal prediction errors) were 32.4% for the *AUC* and 13.6% for the *Cmax*.

**Pharmacodynamic parameters**

**Blood pressure and heart rate** The main results on the average pharmacodynamic parameters are listed in Table 2. The mean time-effect curve for diastolic blood pressure is represented in Figure 3. Blood pressures dropped during treatment: the maximum decrease (systolic / diastolic) was 15.6/10.6 mmHg with the rilmenidine solution (from 111/60 at baseline), and 22.1/14.2 mmHg with the tablet (from 117/63 at baseline). The difference between the two preparations was not statistically significant (95% confidence interval (95% CI) -1.5, 14.4 mmHg systolic, and -0.6, 7.7 mmHg diastolic). The differences in average response (*AUEC* over 12 hours) were also not significant (Table 2).

The maximum effect of the tablet (Table 2) occurred on average 2.9 h (95% CI 0.5, 5.3 h) later for systolic, and 2.5 h (95% CI 1.0, 4.0 h) later for diastolic blood pressure, compared to the solution.

A significantly higher increase in *Emax* of heart rate of 4.6 bpm was observed for the solution (95% CI: 1.2, 7.9 bpm). The time of maximal effect for heart rate was similar for the two preparations.

**Visual analogue scores** Clear differences in times of maximal effect were noted for *VAS* alertness as represented in Table 2. *Tmax* occurred on average at 3.5 h after the administration of the solution, and 5.4 h after the ingestion of the sustained release tablet resulting in an average difference of 1.9 h (95% CI 0.1, 3.7 h) between the two treatments. There were no differences in the other *VAS* factors (mood/calmness).
<table>
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<th>Solution SD</th>
<th>sr Tablet Mean</th>
<th>sr Tablet SD</th>
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**Discussion**

This study shows that sustained release has been achieved for the tablet: maximum levels were lower, levels above 75% of the C<sub>max</sub> were maintained for longer periods, and concentrations were two-fold higher at 24 hours after ingestion. The dose-normalised C<sub>max</sub> for the sr tablet was lower than for the solution, while the corresponding t<sub>max</sub> and t<sub>75</sub> were much longer for the sr tablet. The relative bioavailability of the tablet was 126%, indicating that a slow release formulation could have a favourable absorption profile compared to the solution.

Deconvolution assumes that the only difference between the two modes of drug administration for a subject lies in the *in vivo* dissolution of the tablet. All other pharmacokinetic parameters and processes are assumed identical. Therefore the (between subject) variability in the plasma AUC after tablet administration must be equal to or higher than the AUC after the solution. Dissolution of the tablet can be the only source of additional variability.
However, we found that the \( \text{AUC} \) is more variable after the solution (CV=37%) than after the SR tablet (CV=17%). This can only be attributed to other sources of variability, for instance due to differences in the absorption process. Additional evidence is provided by the fact that average relative bioavailability is larger than 100% which must be due to either differences in the relative absorption process or high variability in pharmacokinetic parameters within a subject. These arguments imply that the basic pharmacokinetic behaviour (absorption, distribution, elimination) is not identical for the two occasions resulting in high variability in the \textit{in vitro} – \textit{in vivo} correlation. As a result, the internal prediction errors were higher than 10% for \( \text{AUC} \) and \( C_{\text{max}} \), thus exceeding the stringent criteria mentioned in the guideline for evaluating the predictability of a level A \textit{in vitro} – \textit{in vivo} correlation (deconvolution followed by comparison of the fraction of drug absorbed to the fraction of drug dissolved). Nevertheless, the percent difference between the mean observed and predicted \( \text{AUC} \) and \( C_{\text{max}} \) were relatively low, indicating that the average \textit{in vitro} dissolution kinetics of the SR tablet is predictive of its \textit{in vivo} characteristics.
Despite a two-fold difference in exposure, no significant difference was observed for the $AUEC_{0-12h}$ between the rilmenidine slow release formulation and solution on blood pressure, heart rate and visual analogue scores. However, the maximum effects occurred significantly later after ingestion of the tablet compared to the solution. Although diurnal influences cannot be excluded without use of a placebo, it seems very likely that these different time effects are due to the ‘slow release’ profile of the tablet, compared to the ‘immediate release’ profile of the solution. The data from this study can be used to optimise the dissolution characteristics of a sustained release preparation. Such a preparation could prolong the antihypertensive activity, while reducing peak-concentration related side effects of rilmenidine.
REFERENCES

