Multiple dose pharmacokinetics and concentration effect relationship of the orally active renin inhibitor remikiren (Ro 42-5892) in hypertensive patients

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- 1 Three double-blind, randomized, placebo controlled, multiple oral dose studies in patients with mild to moderate hypertension were performed to study tolerability, pharmacodynamics and pharmacokinetics of remikiren. Doses of 100–800 mg remikiren or placebo were given over 8 days to altogether 144 patient volunteers. In some cases ($n = 46$) single i.v. doses of 100 mg were administered 4 h after the last oral dose. Plasma remikiren concentrations, plasma renin activity and immuno-reactive renin concentrations were measured. Pharmacokinetic parameters were estimated using model independent techniques and the concentration-effect relationship was evaluated using population pharmacometric methods.
- 2 In most patients no distinct absorption and disposition phase could be identified, since plasma concentrations fluctuated widely over a period of approximately 10 h. Peak plasma concentrations ($C_{\max}$) were achieved within 0.25–2 h postdose. Mean $C_{\max}$ values (on the first and last day of oral treatment) were in the magnitude of 4–6 ng ml⁻¹ (200 mg), 23–27 ng ml⁻¹ (300 mg), 65–83 ng ml⁻¹ (600 mg) and 47–48 ng ml⁻¹ (800 mg). $C_{\max}$ and AUC_{0–t} values were clearly different for different doses within single studies. Intersubject variability in pharmacokinetic parameters was much higher than intrasubject variability. No drug accumulation in plasma was apparent.
- 3 Inhibition of the angiotensin I production rate correlated well with plasma drug concentrations according to the $E_{\max}$ -model. An IC_{50} value of 0.5 ng ml⁻¹ (0.8 nM) was estimated. No correlation between blood pressure changes on the last day of oral treatment and either plasma remikiren concentrations or plasma renin inhibition was found.

Keywords renin inhibitor oral application pharmacokinetics
pharmacodynamics population pharmacometrics

Introduction

Since the introduction of the angiotensin converting enzyme (ACE) inhibitors into clinical practice, it has become evident that inhibition of the renin angiotensin system is an effective means of treating various forms of hypertension [1]. Although the ACE inhibitors are generally well tolerated their use is accompanied by adverse events (e.g. cough and angioedema) that are ascribed to accumulation of bradykinin or substance P which are both inactivated by ACE *in vivo* [2, 3, 4]. In contrast, blocking the renin angiotensin system by inhibition of renin is a highly specific pharmacological

intervention, as angiotensinogen is the only known natural substrate for renin. Therefore, considerable efforts have been made over the last several years to develop orally active, low molecular weight renin inhibitors [5, 6, 7].

Remikiren is a potent and selective inhibitor of primate renin *in vitro* with an IC_{50} of 0.8 nM [8, 9]. Long lasting reduction in blood pressure has been shown in hypertensive marmosets and squirrel monkeys [8] as well as in hypertensive patients [10, 11, 12] following both i.v. and oral administration. Pharmacokinetic data obtained

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in healthy volunteers [13, 14, 15] showed a high systemic plasma clearance (900 ml min^{-1}), a large volume of distribution (70 l) and a very low oral bioavailability (around 1%), which is probably due to the high liver first-pass extraction of the compound. The between subject variability of the oral pharmacokinetics of remikiren was high in all studies. There was, however, a very good correlation between the drug concentrations and the pharmacodynamic effect of the net inhibition of plasma renin activity in the plasma compartment in healthy male volunteers [14]. These *in vivo* results on plasma renin inhibition were in good agreement with the results found *in vitro* [8].

Three clinical pharmacology studies were performed in order to evaluate the tolerability and the haemodynamic effects of remikiren in relation to its pharmacokinetics in patients with mild to moderate hypertension. This manuscript summarizes the results on the multiple dose pharmacokinetics and the correlation between drug concentrations and the pharmacodynamic effects of remikiren. Detailed results of tolerability and haemodynamic effects, especially effects on blood pressure have in part already been published elsewhere [12].

Methods

Study design and subjects

Study A This was a multicentre, double-blind, randomized, placebo controlled, parallel group, multiple ascending dose study designed to evaluate mainly the tolerability and in addition haemodynamic effects and pharmacokinetics of remikiren. Seventy male patient volunteers with mild to moderate essential hypertension aged 30 to 64 years participated. Five subjects dropped out for non-medical reasons and four subjects were not eligible due to violation of the inclusion/exclusion criteria. After a single-blind placebo run-in period of 8 days, eligible patients (sitting diastolic blood pressure was between 100–115 mm Hg) were randomized to receive either remikiren ($n = 12$ per dose level) or placebo ($n = 4$ per dose level) once daily for 8 days. Doses of 100 mg ($n = 12$ evaluable), 200 mg ($n = 12$ evaluable), 400 mg ($n = 11$ evaluable) and 800 mg ($n = 10$ evaluable) remikiren were administered always on an empty stomach as an oral solution in orange juice.

Study B This was a multicentre, double-blind, randomized, placebo controlled, parallel group, multiple dose study designed to investigate the tolerability, haemodynamic effects and pharmacokinetics of remikiren. Fifty-one patient volunteers (26 male, 25 female) with mild to moderate essential hypertension aged 24 to 69 years participated (one of these patient volunteers dropped out during the placebo run-in period, one patient was excluded from analysis due to violation of the inclusion/exclusion criteria). Following a 3-week wash-out period (without antihypertensive medication), all patients received oral placebo for 8 days (run-in period), after which those patients whose sitting diastolic blood pressure was between 100–115 mm Hg

were randomized to receive, once daily for 8 days, placebo ($n = 16$) or active treatment ($n = 17$ per dose level) comprising either 300 mg or 600 mg remikiren. The drug was given always on an empty stomach as 100 mg capsules. On the last day of this oral treatment period, all patients receiving active treatment received a single i.v. dose of 100 mg remikiren, 4 h after the oral dose. Patients in the placebo group received i.v. placebo at this time.

Study C This was a single centre, double-blind, randomized, placebo controlled, parallel group, multiple dose study designed to investigate haemodynamic effects, tolerability and pharmacokinetics of remikiren. Twenty-four male patient volunteers with mild to moderate essential hypertension aged 38 to 64 years participated. After a 3-week wash-out period (without antihypertensive medication) patients entered a 1-week single-blind placebo run-in period. Those patients whose sitting diastolic blood pressure was between 95–114 mm Hg at the end of this run-in period were randomized to receive, once daily for 8 days, either placebo ($n = 12$) or 600 mg remikiren ($n = 12$). The drug was given as 100 mg capsules 1–2 h following breakfast each day. On the last day of this oral treatment period, 4 h after the oral dose, patients were randomized again in a double-blind fashion to receive either i.v. placebo ($n = 6$ of each group) or 100 mg i.v. remikiren ($n = 6$ of each group).

In all three studies sitting blood pressure, heart rate and ECG were measured repeatedly, blood samples for routine clinical laboratory analyses were taken repeatedly and adverse events were recorded throughout the study. Blood samples were repeatedly taken up to 24 h post-dose for determination of biochemical parameters and measurements of plasma remikiren concentrations on the first and last days of the oral treatment period. All patients gave their written informed consent before the trials started.

Sample analysis

All blood samples were collected into vacutainer tubes containing EDTA as anticoagulant, immediately cooled on ice, centrifuged, and plasma was separated and stored at -20°C until analyzed.

Remikiren concentrations Three different analytical methods were used for analysis of plasma remikiren concentrations from the three clinical studies. All three methods have been cross examined using spiked plasma samples of various concentrations (0.50, 1.00, 2.50, 10.0, 25.0, 80.0, 200 ng ml^{-1}) as well as 132 authentic plasma samples from a clinical study with measured concentrations ranging from 0.38 to 137 ng ml^{-1} . All spiked samples were analysed 3 to 8 times and all authentic samples were analysed in the single mode with each method. No evidence for differences in analytical results between the assays over their valid concentration range has been found [16]. The validity of each analytical run was guaranteed by simultaneous analysis of independently prepared quality control samples of various concentrations.

The first method available was a specific h.p.l.c. method employing reversed phase chromatography on a nucleosil C₁₈ 3 µm column with fluorescence detection following precolumn derivatization of remikiren and the internal standard (Ro 42-4661) with fluorenylmethylchloroformate [11]. The limit of quantification was 1.3 ng ml⁻¹. The interassay coefficients of variation were in the range of 1.5% to 11.5%. The mean inaccuracy ranged from -9.8% to +9.9%. This assay was used to analyze all samples of study C as well as the 800 mg dose samples of study A.

The second method used was a specific h.p.l.c. method employing reversed phase chromatography on a C₁₈ column and post-column irradiation using a Beam Boost® followed by coulometric oxidative electrochemical detection at +550 mV vs a H₂/H⁺ electrode [17]. The limit of quantification was 0.2 ng ml⁻¹. The interassay coefficients of variation were in the range of 2.7% to 13.9%. The mean inaccuracy ranged from -15.0% to +1.7%. The 200 mg dose samples of Study A were analyzed using this method.

The third method used was a specific protein binding assay modified from the assay of Cumin *et al.* [18]. Briefly, plasma samples were incubated together with recombinant human renin and [³H]-labelled remikiren followed by the addition of dextran coated charcoal and subsequent centrifugation. The supernatant was analyzed for protein bound radioactivity by liquid scintillation counting. The limit of quantification was approximately 0.4 ng ml⁻¹ dependent upon the daily performance of the assay. The interassay coefficients of variation were in the range of 6.4% to 14.5%. The mean inaccuracy ranged from -2.6% to +4.6%. All samples of study B were analyzed using this method.

Plasma renin activity Plasma renin activity (PRA) was measured by trapping radioimmunoassay using the standard generation rate of angiotensin I from endogenous angiotensinogen in the presence of angiotensin I antibodies as trapping reagents for angiotensin I [19, 20, 21]. Samples were incubated at 37° C at pH 7.4 and angiotensin I formation was determined. The interassay coefficients of variation were in the range of 15.0% to 26.3%. The mean inaccuracy ranged from -34.5% to -5.6%.

Immunoreactive renin Immunoreactive renin (IRR) was measured by radioimmunometric assay using monoclonal antibody 4 G 1 (Diagnostics Pasteur Kit) [22]. The interassay coefficients of variation were in the range of 3.6% to 13.0%. The mean inaccuracy ranged from -9.8% to -0.1%.

Data analysis

Pharmacokinetic analysis Pharmacokinetic evaluation was performed using model independent techniques. The pharmacokinetic parameters estimated were peak plasma concentrations ($C_{\max}$), time to reach $C_{\max}$ ($t_{\max}$) and area under the curve from time 0 to the time when the last sample with measurable concentrations was taken (AUC_{0-t}). $C_{\max}$ and $t_{\max}$ values following oral administration were taken directly from the observed

plasma concentration versus time data. AUC_{0-t} was estimated using the linear trapezoidal rule [23] up to the last measured concentration value.

Concentration-effect modelling Inhibition of the angiotensin I production rate at time t (APR) was calculated as:

$$\text{APR-Inhibition} = \left(1 - \frac{\text{PRA}_t/\text{PRA}_0}{\text{IRR}_t/\text{IRR}_0}\right) \cdot 100\%$$

where the subscript 0 indicates values obtained before administration of the drug. PRA_t and IRR_t values were not corrected for placebo. The inhibition of the angiotensin I production rate was related to the plasma concentration of unchanged remikiren using the Hill equation:

$$\text{APR-Inhibition} = \frac{I_{\max} \cdot C_p^n}{IC_{50}^n + C_p^n}$$

where $I_{\max}$ is the maximal inhibition (effect), IC_{50} the concentration required to produce 50% of the maximal effect, n the Hill coefficient, a measure for sigmoidicity, and C_p the plasma concentration of remikiren. Estimates for $I_{\max}$ and IC_{50} (fixed effects) and for the random effects (intersubject variability) linked to the fixed effects as constant coefficients of variance, were obtained using double precision NONMEM version III level 1.2 [24, 25]. Data input to and data retrieval from NONMEM and graphical display were facilitated by an RS/1 shell developed at Fa. Hoffmann-La Roche.

The relationship between plasma concentrations and blood pressure changes and between plasma renin inhibition (PRA and APR) and blood pressure changes were evaluated analogous. Thereby data on blood pressure were evaluated using two approaches. First they were corrected for placebo by simple mathematical subtraction of the corresponding mean values of the placebo treated control group at the various time points for each study and for the second approach they were used uncorrected. Data on PRA or APR were in no case corrected for placebo.

Statistical analysis Descriptive statistics were applied and means and standard deviations (s.d.) were calculated for the pharmacokinetic parameters. The estimated parameters $C_{\max}$ and AUC_{0-t} were compared between the first and last oral dose and between the different doses within the same study using the two-sided paired (or unpaired, as appropriate) t -test procedure. A level of $P < 0.05$ was considered as statistically significant.

Results

The estimated pharmacokinetic parameters are summarized in Table 1. Representative individual and mean plasma concentration-time curves of remikiren for study A are shown in Figure 1.

Table 1 Pharmacokinetic parameters of oral remikiren in patients with mild to moderate essential hypertension from three different clinical pharmacological studies

Study	Dose (mg)	t_{max} (h)		C_{max} (ng ml ⁻¹)		AUC_{0-t} (ng ml ⁻¹ h)	
		Day 1	Day 8	Day 1	Day 8	Day 1	Day 8
A	200	0.4 ± 0.1 (0.3–0.5)	1.5 ± 2.5 (0.5–8.0)	6.3 ± 8.0 (1.6–26.8)	4.1 ± 4.2 (0.6–14.1)	17.0 ± 19.3 (2.0–66.2)	14.1 ± 13.6 (2.9–43.5)
	800	0.5 ± 0.4 (0.3–1.0)	0.7 ± 0.4 (0.3–1.5)	47.3 ± 35.3 ³ (7.3–87.4)	47.6 ± 41.0 ³ (8.7–152.2)	69.9 ± 54.3 (24.0–136.5)	65.9 ± 50.3 (26.5–177.6)
B	300	0.7 ± 0.5 (0.4–2.0)	0.8 ± 0.5 (0.4–2.0)	26.5 ± 27.2 (3.4–111)	22.6 ± 25.4 (2.1–89.7)	40.8 ± 41.4 (4.2–144.4)	— ¹
	600	0.8 ± 0.4 (0.3–2.0)	0.8 ± 0.5 (0.4–2.0)	62.2 ± 39.2 ³ (5.0–151)	65.0 ± 85.3 ³ (1.7–351)	143.5 ± 128.0 ³ (6.3–553.3)	— ¹
C	600	1.0 ± 0.9 (0.5–4.0)	0.9 ± 0.4 (0.5–1.5)	83.0 ± 164.4 (5.5–623.1)	76.9 ± 81.8 (14.5–334.5)	95.5 ± 144.3 ² (12.9–543.3)	88.1 ± 83.3 ² (35.4–252.4)

Data are presented as mean ± s.d. (range).

¹Not applicable as all subjects received an i.v. dose 4 h after the last oral dose.

²Only data of those subjects who received i.v. placebo 4 h after the last oral dose are presented.

³ $P < 0.05$ if compared with lower dose in the same study.

A large intersubject variability was observed in all three studies. Peak plasma concentrations varied between 15-fold (study A, 800 mg dose) and 208-fold (study B, 600 mg dose) within one dose level measured on the same study day. Similarly, AUC_{0-t} values varied between 7-fold (study A, 800 mg dose) and 88-fold (study B, 600 mg dose) within one dose level. Intrasubject variability was much smaller as a comparison of the data from the first and the last days of active treatment shows. For example, C_{max} varied maximally 11-fold (study B, 300 mg dose) and AUC_{0-t} varied maximally 3-fold (study A, 800 mg dose and study C, 600 mg dose) within the same subject. No accumulation of drug in plasma during the 8 days of once daily treatment was apparent.

Overall, highly fluctuating plasma concentration-time profiles were observed and no typical absorption and elimination phase could be discerned in most patients. Therefore, no terminal half-life could be estimated and no extrapolation of AUC from the time of the last measurable plasma concentration to infinity could be performed. The truncated AUC values calculated from plasma concentration data could not be compared, as the limit of quantification of the three analytical methods was different (i.e. from 0.2 ng ml⁻¹ with the h.p.l.c. method employing electrochemical detection to 1.3 ng ml⁻¹ with the h.p.l.c. method employing fluorescence detection). In study B a statistically significant difference in the AUC values of the 300 mg and 600 mg dose was found. The C_{max} values achieved with different doses in each study also differed in a statistically significant way and were thus dose dependent. t_{max} values varied between 0.25 and 2 h but tended to be in the lower range between 0.25–1 h in most cases.

Two trends which were, however, not statistically significant, were recognized. First, with the solid formulation used for studies B and C obviously higher plasma levels and thus a higher bioavailability was achieved than with the oral solution used for study A. And second, no difference in pharmacokinetic para-

eters between male and female patient volunteers in study B was obvious.

The relationship between inhibition of the angiotensin I production rate and plasma remikiren concentrations was well described by the E_{max} -model with the Hill coefficient set to 1 (Figure 2). I_{max} was estimated as 90.3% (CV = 1.4%). Its interindividual variability of 7.4% was very small. IC_{50} was 0.5 ng ml⁻¹ (CV = 32.3%) but could be less precisely estimated. Its interindividual variability was 111%. No statistical significant correlation between blood pressure changes and plasma remikiren concentrations or plasma renin inhibition (whether corrected for changes in immunoreactive renin concentrations or not) were detected independent whether blood pressure data were corrected for placebo measurements in the respective control group of each study or not (see Figure 3).

Discussion

Various analytical methods for quantitative analysis of plasma remikiren concentrations have been developed during the last years. They were optimized with respect to different parameters such as high sample throughput, low limit of quantification and/or easy sample handling. The three studies reported in this paper were performed over a time period of 1–2 years during which the three described analytical methods were available at different time points. Always the best assay which was available at a given time when samples were available was used. In some instances, samples from lower doses were analyzed later in time when a more sensitive analytical assay was available. Because of the different limits of quantification of the assays used, the results of the three studies concerning bioavailability cannot be compared in terms of AUC, but only in terms of C_{max} .

The pharmacokinetic parameters of orally administered remikiren reported in this paper showed a high

Figure 1 Plasma remikiren concentration-time profiles from study A: individual profiles on the first (a) and last day (b) of the 200 mg dose treatment and the first (c) and last day (d) of the 800 mg dose treatment and mean profiles for both doses and both treatment days (e). Note that the y-axis for (a), (b) and (e) is different from (c) and (d).

intersubject variability. This was already observed in healthy volunteers and was expected as a consequence of the high liver first-pass extraction of the compound [14]. Despite this high variability, there was a clear dose dependency in $C_{\max}$ (study A and B) and AUC (study B). Dose dependent pharmacokinetics of the drug has already been reported over a single oral dose range of 400–1600 mg in healthy volunteers [13, 14]. No drug accumulation in plasma over the 8 days of oral treatment was apparent. It is not known why the plasma concentrations of remikiren following oral administration fluctuated with time in that irregular fashion not exhibiting clear absorption and disposition patterns. Reasons for this behaviour could be e.g. a discontinuous dissolution and repeated precipitation of remikiren in parts of the gastrointestinal tract due to its low solubility at high pH or the existence of enterohepatic recirculation of

the drug. Although fast and extensive biliary excretion of drug and metabolites was shown in animals (Richter W, Jauch G, Coassolo Ph., personal communication, 1992), there is no direct evidence for enterohepatic recirculation of drug or drug related material in animals or in man so far.

A low oral bioavailability in man has been described for most experimental renin inhibitors, e.g. 0.5% for enalkiren [26] and < 1% for CGP 38560A [27]. For remikiren this is the consequence of low absorption and —more important—of high liver first-pass effect [14]. Hence, efforts are being made to synthesize non-peptidic renin inhibitors with higher oral bioavailability [28]. In spite of the low bioavailability remikiren should be an efficacious drug in terms of plasma renin inhibition following oral administration, since plasma concentrations above the *in vitro* IC_{50} of 0.8 nM (0.5 ng ml⁻¹) were

Figure 2 Correlation between inhibition of the angiotensin I production rate (APR) and plasma concentrations of remikiren. The dotted line is a smoothed line through the observed individual data and the dashed line is a smoothed line through the model predicted individual data. The single line ('fitted line') represents a simulation for an average subject from the studied population using the estimated population means of the parameters $I_{\max}$ and IC_{50} (Hill coefficient = 1).

Figure 3 Plot of the changes in diastolic (SDBP) and systolic (SSBP) blood pressure vs remikiren plasma concentrations (a, b) and the inhibition of angiotensin I production rate (c, d). Note that blood pressure data in these plots are not corrected for placebo.

maintained over approximately 12 h following relatively low doses (200 mg). In fact, a dose dependent prolonged decrease in plasma renin activity (PRA) and transient increase in immunoreactive renin (IRR) was observed *in vivo* in healthy volunteers [11, 13, 14, 29, 30] and in hypertensive patients [12]. In the patients of the reported studies, an excellent correlation between the inhibition of the angiotensin I production rate (APR) *in vivo*, which presents the net inhibition of plasma renin (PRA corrected for IRR), and the plasma concentrations of remikiren was found. The relationship was perfectly described by the $E_{\max}$ -model with a surprisingly small interindividual variability (Figure 2). The estimated values for $I_{\max}$ (90.3%) and IC_{50} (0.5 ng ml^{-1}) were in good agreement with the results found in healthy volunteers following single oral doses ($I_{\max} = 95\%$, $IC_{50} = 1.7 \text{ ng ml}^{-1}$) [14] and also with results from *in vitro* studies ($IC_{50} = 0.5 \text{ ng ml}^{-1}/0.8 \text{ nM}$) [8]. Therefore, it can be concluded that the inhibition of plasma renin activity by remikiren is well predictable from the achieved plasma drug concentrations. If the inhibition of plasma renin is responsible for reducing blood pressure in hypertensive patients then a relationship between these two parameters is expected. However, no correlation between changes in blood pressure and plasma renin inhibition or plasma drug concentrations was found. Over the past years, the existence of specific tissue renin angiotensin systems in parallel to the plasma renin angiotensin system and their importance in the regulation of blood pressure have been amply discussed [31, 32]. Recent findings with remikiren in animals [8] and hypertensive patients [10] have supported the hypothesis that an extravascular renin angiotensin system is involved in the regulation of blood pressure. Remikiren is widely distributed in almost all organs and retained for a long time in some tissues as e.g. the kidney, salivary glands and the thyroid in various animal species (Richter W, personal communication, 1992). Therefore and because of its high volume of distribution in man [14], it could be speculated that drug concentrations in the tissues

would be sufficiently high to contribute to the haemodynamic effects of remikiren by inhibition of tissue renin in addition to inhibition of plasma renin. The lack of correlation between the observed changes in blood pressure and plasma drug concentrations could be explained on the basis of various pharmacokinetic but also on the basis of various pharmacodynamic aspects which are summarized in the following paragraph. For example, one could argue that a) steady state conditions have not yet been achieved with a treatment duration of 8 days and/or b) not high enough tissue (effect compartment) concentrations of remikiren have been achieved to inhibit the tissue renin angiotensin system or c) plasma renin has not been inhibited for a long enough period of time to lead to a blood pressure reduction or d) the circadian variability of blood pressure has not been taken into account for because e) no data on placebo treatment in individual patients have been collected. These hypotheses will have to be tested in future experiments with a longer duration of treatment.

In summary, remikiren showed highly variable pharmacokinetics in hypertensive patients following oral administration. No accumulation of drug in plasma over the 8 day treatment period was apparent. The plasma concentration-time data did not show typical smooth absorption and disposition phases. Peak plasma concentrations were achieved within a relatively short time and concentrations above the IC_{50} of 0.8 nM were maintained over several hours following relatively low doses. Effects on plasma renin inhibition were well predictable from the plasma concentrations of remikiren. However, no correlation between changes in blood pressure and either plasma drug concentrations or PRA inhibition was found after 8 days of treatment.

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