Renal and systemic effects of continued treatment with renin inhibitor remikiren in hypertensive patients with normal and impaired renal function

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Abstract
Background. Remikiren is an orally active renin inhibitor with established antihypertensive efficacy. As a single dose it induces renal vasodilatation, suggesting specific renal actions. Data on the renal effects of continued treatment by renin inhibition are not available, either in subjects with normal, or in subjects with impaired renal function.

Methods. The effect of 8 days of treatment with remikiren 600 mg o.i.d. on blood pressure, renal haemodynamics, and proteinuria was studied in 14 hypertensive patients with normal or impaired renal function. The study was conducted on an ambulatory in-hospital basis and was designed in a single-blind, longitudinal order.

Results. Remikiren induced a significant peak fall in mean arterial pressure of 11.2 ± 0.8%, with corresponding trough values of −6 ± 0.8%. This fall was somewhat more pronounced in the patients with renal function impairment (−13.3 vs −9.6%; P < 0.01). Glomerular filtration rate remained stable, whereas effective renal plasma flow increased from 301 ± 35 to 330 ± 36 ml/min/1.73 m² (P < 0.05). Filtration fraction and renal vascular resistance fell by 10 ± 2% and 15 ± 2% respectively (both P < 0.01). Remikiren induced a cumulated sodium loss of −82 ± 22 mmol and a positive potassium balance of 49 ± 9 mmol (both P < 0.01). During remikiren, proteinuria fell by 27% (range −18 to −38%; P < 0.01) in the patients with overt proteinuria at onset (n = 6). In the remainder of the patients albuminuria fell by 20% (range −1 to −61%, P < 0.05). No side-effects were observed.

Conclusions. Continued treatment with remikiren induced a sustained fall in blood pressure, renal vasodilatation, negative sodium balance, and a reduction in glomerular protein leakage. These data are consistent with a renoprotective potential of renin inhibition.

Keywords: chronic renal failure; hypertension; proteinuria; renal haemodynamics; renin inhibition

Introduction
The renoprotective potential of blocking the renin–angiotensin system (RAS) by angiotensin-converting enzyme inhibitors (ACEi) in renal patients has been demonstrated in several studies [1,2]. Whether blockade of the RAS at other levels, such as blockade of the AT₁ receptor [3] will convey long-term renoprotection as well is still under investigation. Renin inhibitors, that block the first and possible rate-limiting step of activation of the RAS, theoretically have the advantage of a high specificity. These agents lack the decreased breakdown of bradykinins as well as the reactive rise in angiotensin II (angII) associated with ACEi and AT₁-receptor blockers respectively. When administered systemically, renin inhibitors reduce blood pressure in healthy volunteers and hypertensive patients more or less similarly to ACEi [4–7]. In spite of the low oral bioavailability several studies reported clear-cut haemodynamic effects of remikiren after oral administration [8–11].

The renal haemodynamic actions of renin inhibitors so far have been consistent with a renoprotective potential. Single-dose renin inhibition induced renal vasodilatation in healthy volunteers as well as essential hypertensive patients [11–14]. Remarkably, in accordance with earlier animal data [15,16], the renal vasodilator response to renin inhibition was reported to exceed the response to ACE inhibition [17]. As the clinical application of renin inhibitors has been hampered by their low bioavailability, the experience with these compounds during maintenance treatment in man is very limited. We found previously that a single oral dose of the renin inhibitor remikiren lowered blood pressure, induced renal vasodilatation, and lowered micro-albuminuria in patients with essential hypertension and normal renal function [11]. No data are

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available thus far on the renal effects of continued treatment with remikiren, or on the renal effects of renin inhibition in patients with renal function impairment and proteinuria. In the present study, therefore, we report on the renal and systemic effects of continued treatment for 8 days the renin inhibitor remikiren in hypertensive patients with normal renal function, as well as in patients with impaired renal function and proteinuria.

Subjects and methods

Patients and protocol

Fourteen caucasian patients with mild to moderate hypertension (diastolic blood pressure between 90 and 115 mmHg) were included. Patients had either essential hypertension with normal renal function (creatinine clearance > 90 ml/min; n = 8, all male) or impaired renal function due to non-diabetic renal disease (a stable creatinine clearance < 60 ml/min; n = 6, two female, four male). No other clinical relevant target organ damage was allowed. Excluded were patients with other secondary forms of hypertension, patients overweight with more than 120% of their ideal body weight (Metropolitan Insurance Life Table), patients with a history of alcohol or drug abuse, and women of childbearing potential or taking birth-control pills. All subjects gave their informed consent and the study was approved by the Ethical Committee of the Hospital.

Median age of the patients was 53 years (range 32–69). The median body mass index at baseline was 26.1 kg/m² (95% CI 24.8–27.5). In the patients with essential hypertension, creatinine clearance at entry was 120 ± 5 ml/min (range 102–149 ml/min). In the renal patients it was 42 ± 4 ml/min (range 32–58). Histological diagnoses were glomerulosclerosis (3); membranous glomerulopathy (2), and IgA nephropathy (1). Systolic blood pressure at entry was 154 ± 2 mmHg (range 130–161) and diastolic blood pressure was 100 ± 2 mmHg (range 90–111); these values were similar for the essential hypertensives and the renal patients. In the essential hypertensives median albuminuria at entry was 13.3 mg/24 h (range 5.5–30.1 mg/24 h). In the renal patients nephrotic range proteinuria was present, with a median of 10.2 g/24 h (range 6.6–13.1 g/24 h) at entry.

The study was performed on an ambulatory in-hospital basis, and was designed in a single-blind, longitudinal order. All antihypertensives had been withdrawn at least 3 weeks prior to the study, with the exception of one proteinuric patient in whom diuretic treatment was necessary. The dose of the diuretic was kept constant throughout the protocol. This patient was excluded from the analysis of electrolyte balance. After the screening and wash-out period the patients were hospitalized for 18 days. During hospitalization they received a diet containing 50 mmol sodium, 100 mmol potassium, 60 g protein, and 2500 ml fluids daily.

After a 5-day run-in period in the hospital, the patients were studied during a 5-day baseline period without treatment. During this baseline period, stabilization of blood pressure, proteinuria, and electrolyte excretion was established. Subsequently, remikiren treatment (600 mg orally o.i.d., administered each day at noon) was given for 8 days. Twenty-four-hour urine was collected daily for determination of sodium, potassium, creatinine, urea, and protein. Blood pressure was measured daily between 11 and 12 a.m., at 5-min intervals, in the supine position. In addition, 48 h continuous ambulant blood pressure recordings were performed before treatment and on the fifth/sixth day of renin inhibition. Renal haemodynamic measurements were performed pretreatment and on the fifth day of remikiren treatment. On the first and the last treatment day timed blood samples were drawn for measurement of plasma renin activity (PRA), immunoreactive renin (irR), and angII.

Methods

Blood pressure was measured by an automatic non-invasive device: Dinamap®, Criticon Inc, Tampa Florida, and the Spacelabs® respectively. Mean arterial pressure (MAP) was calculated as diastolic blood pressure plus one-third of the pulse pressure. For each hour the mean value was calculated. Urinary sodium, potassium and creatinine were measure by a standard autoanalyser technique (SMA-C, Technicon®, Tarrytown, NY). Urinary protein was measured by the pyrogallol red–molybdate method. Blood samples for measurement of irR and PRA were collected into Vacutainer tubes at room temperature with EDTA as an anticoagulant. Blood samples for angII were collected into pre-chilled Vacutainer tubes containing an inhibitor cocktail consisting of EDTA, the ACEI cilazaprilat, and the renin inhibitor Ro 42–5892, in order to prevent the formation of angII during processing. All samples were immediately separated; then plasma was immediately stored at −20 until analysis. The irR was determined using the renin IRMA Pasteur kit. This method has a coefficient of variation of 15.9% (inter-assay) and 6.6% (intra-assay, both at 31 pg/ml). PRA was measured as the quantity of generated angI measured by RIA (Riannen® Ang I RIA kit). AngII was determined by the Nichols Institute Diagnostics Ang II RIA, with a coefficient of variation of 5.1% (inter-assay, mean value 31 pg/ml) and 4% (intra-assay, mean value 42 pg/ml). During analysis plasma was first separated from plasma proteins by ethanol extraction. The extracted plasma angII was measured in a sensitive and specific competitive protein-binding radioimmunoassay.

The lower limit of detection is 3.6 pg/ml. Circulating levels of remikiren were determined by the radioinhibitor assay of Cumin et al. [18], using 3H RO 42–5892 as a tracer. Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were measured as the renal clearances of constantly infused [125]iothalamate and [131]hippuran respectively. Correction for urine collection errors was applied as described previously [19]. The day-to-day coefficient of variation of this method is 2.2 and 5% respectively. Values are normalized for body surface area. Filtration fraction (FF) is calculated as the ratio of GFR/ERPF. Renal vascular resistance (RVR) is calculated as the ratio of MAP and ERPF.

Data analysis

Results are presented as means ± SEM. Data sets that are not normally distributed are presented as medians and ranges. Sodium and potassium balance are calculated by subtracting 24-h urinary excretion from the individual mean excretion during the last 2 pretreatment study days. Data on hormonal responses, blood pressure, renal haemodynamics, and electrolyte balance are presented for all patients taken together. Data on the responses of proteinuria and albuminuria are presented for the renal patients and the essential hypertensives separately. Statistical analysis was performed by a paired, non-parametric ANOVA (Friedmann) for repeated measurements, followed by Dunn’s correction for multiple compar-
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Results

The efficacy of remikiren in blocking the RAS, as well as the drug levels, are shown in Figure 1. PRA fell maximally within 30 min after the first dose of remikiren and remained suppressed during the 12 h following. At day 8, trough values were 65% below (range 2–87%, \( P < 0.05 \)) the pre-treatment value on day 1, indicating inhibition of PRA throughout the day. After dosing, a further decrease to almost undetectable levels occurred. AngII fell up to 6 h after the first dose, but the trough value at day 8 was not significantly different from the pre-treatment value. The level of irR increased until 4 h after the first dose, whereas the trough value on day 8 was increased by 30% as compared to pretreatment values (range 35 to 82%; \( P < 0.05 \)). Remikiren plasma levels reached their peak within 30 min after oral administration both on the first (median \( C_{\text{max}} \) 60.2, range 6.1–252 ng/ml) as well as the last day of treatment (\( C_{\text{max}} \) 54.2, range 32.7–342 ng/ml). Remikiren was rapidly cleared from the plasma thereafter. No differences in pre-treatment hormonal parameters or in the humoral response to treatment were observed between the renal patients and the essential hypertensive patients.

In Figure 2 the time course of mean blood pressure at trough (\( n = 14 \)) is given. It shows that MAP fell significantly, from 108 ± 2 to 96 ± 2 mmHg. The effect on diurnal blood pressure profile is given in Figure 3. It shows that MAP is reduced throughout 24 h and

![Fig. 2. Mean arterial pressure (mean ± SEM) during baseline and 8 days treatment with remikiren, 600 mg o.i.d. *\( P < 0.05 \) vs mean of last 2 baseline days.](image)

![Fig. 3. Mean arterial pressure (MAP, mmHg; mean ± SEM, upper panel) and heart rate (HR, beats per minute, lower panel) during 2 x 24 h, both on days −1 and −2 of baseline (continuous lines) as well as on days 6 and 7 during remikiren treatment (broken lines).](image)
that heart rate did not change. The fall in blood pressure was similar for systolic and diastolic blood pressure. The peak fall in MAP was \(-11.2 \pm 0.8\%\) (range \(-7.5\) to \(-17.4\), average of a 4-h period) on day 7 with a corresponding trough value of \(-6 \pm 1\%\) (range \(-0.1\) to \(-16.2\)). This resulted in a trough to peak ratio of 0.53 \pm 0.11. The fall in blood pressure was more pronounced in the chronic renal failure patients (\(-13.3 \pm 1.1\%\)) than in the essential hypertensive patients (\(-9.6 \pm 0.7\%\); \(P<0.01\), Mann–Whitney).

The renal haemodynamic effects are shown for individual patients in Figure 4. It shows that GFR remained stable, with a mean value of 65 \pm 9 ml/min/1.73 m\(^2\) before treatment, and a mean value of 63 \pm 8 ml/min during remikiren. ERPF increased from 301 \pm 35 to 330 \pm 36 ml/min (\(P<0.05\)). As a result, FF fell by 10 \pm 2\% (range 1–19\%; \(P<0.01\)). Mean MAP was lowered from 106.7 \pm 2% to 97.9 \pm 1.9 mmHg (\(P<0.001\)) during the renal haemodynamic measurements, accordingly a decrease in RVR of 15 \pm 2\% (range 2–30\%) could be calculated (\(P<0.01\)).

Remikiren exerted significant effects on the renal excretion of sodium and potassium (Figure 5). During the pre-treatment period excretion had stabilized in all patients, with a pre-treatment value of 37 \pm 2 (range 24–44) mmol/24 h for sodium and of 88 \pm 1 (range 82–100) mmol/24 h for potassium, showing excellent diet compliance. During remikiren treatment, a cumulative sodium loss of \(-82 \pm 22\) mmol (range \(-187\) to \(-45\)) mmol occurred, with a positive potassium balance of 49 \pm 9 mmol (range 3–91) (both \(P<0.01\)). This negative sodium balance corresponded to a decrease in body weight of 0.9 kg (range –1.6 to 0.4 kg; \(P<0.05\)). During these 8 days, a transient fall in aldosterone was observed with a return to values not different from baseline as of day 4 (Figure 5). In the chronic renal failure patients both the sodium loss (\(-98 \pm 31\) vs \(-72 \pm 32\) in essential hypertensive patients) and the potassium retention (60 \pm 13 vs 42 \pm 11 in essential hypertensive patients) tended to be somewhat more pronounced, although not significantly so (both \(P<0.1\), NS).

The effect of remikiren on proteinuria in the six renal patients is shown in Figure 6, together with the effect on MAP in these patients. During the in-hospital run-in period, proteinuria had gradually fallen to values below those obtained at entry, with stabilization during the 5-day baseline period. Median baseline value was 5.8 g/24 h (range 2.4–11.9). Remikiren induced a gradual and significant fall in proteinuria, with a maximal decrease of 27\% (median value, range \(-38\) to \(-18\%\)) (\(P<0.01\)). In the essential hypertensive patients a reduction in renal protein leakage was found as well. Median pre-treatment albuminuria after stabilization in hospital was 7.8 mg/24 h (range 4.3–10.4 mg/24 h); after 8 days treatment with remikiren, albuminuria had fallen by a median of \(-20\%\) (range \(-61\) to \(-1\%\), \(P<0.05\)).

No significant effects on serum electrolytes, albumin or cholesterol were observed during treatment. No side-effects were experienced by any of the patients during this study.

**Fig. 4.** The individual effects of remikiren on glomerular filtration rate (GFR, ml/min/1.73 m\(^2\)) and effective renal plasma flow (ERPF, ml/min/1.73 m\(^2\)).

**Fig. 5.** Effects of remikiren on cumulative sodium balance (upper panel, mmol), cumulative potassium balance (middle panel, mmol) (both mean \pm SEM), and plasma aldosterone concentration (lower panel, PAC, pmol/l; mean and 95\% CI). *\(P<0.05\) vs baseline.
immediately after dosing as well as the rapid clearance suggests that efflux lead to plasma accumulation of the drug. This was not likely to indicate renoprotective potential, as reduction—kiren despite undetectable plasma drug levels [8] within the body [23]. This would explain the sustained ment. In renal patients this renal haemodynamic pro targets tissue for remikiren and that retention of the ers [3,25]. Interestingly, this renal pro kinetic pro—fl—observed, associated with a positive potassium balance. re in whom albuminuria fell signifi—cant rise in ERPF despite the fall in blood pressure. Remikiren induced renal vasodilatation, with a signi—cant blood-pressure response with renin inhibition. Several factors could account for the more pronounced blood pressure response in our study. These include the use of a different remikiren compound (later generation), the in-hospital study design, the use of a sodium—restricted diet, and finally the different patient population. Interestingly, in our population the six patients with renal function impairment had a more pronounced blood-pressure response than the essential hypertensive patients. Whether this is due to pharmacokinetic factors (i.e. higher drug levels) or to pharmacodynamic factors (such as a greater contribution of the RAS in vasomotor tone) cannot be ascertained from the present data. Hormonal parameters were similar for the renal patients and the essential hypertensive patients, but this does not exclude differences in activity of tissue RAS.

Our study is the first to provide data on the renal effects of continued treatment with renin inhibition. This allowed an assessment of the effects on sodium and potassium balance. Remikiren induced a net negative sodium balance—in spite of the fall in blood pressure—and a concomitant positive potassium balance in both essential hypertensive and renal patients. The combination of sodium loss and potassium gain was also reported for ACE inhibition, and is usually attributed to a suppression of aldosterone. The gradual return to baseline values of aldosterone after its initial decline—a feature that we have previously reported to occur with ACE inhibition as well [24]—presumably reflects stimulation of aldosterone by the positive potassium balance, as the persistently elevated PRA indicates an ongoing drug effect on RAS activity.

Remikiren induced renal vasodilatation, with a significant rise in ERPF despite the fall in blood pressure. The renal haemodynamic profile, with a fall in FF, suggests that efferent arteriolar vasodilatation predominates over afferent vasodilatation. Such renal vasodilatation may well have facilitated renal sodium loss. The effects of remikiren on renal haemodynamics are in line with those with other modes of blocking the RAS, such as ACE inhibitors and AT1-receptor blockers [3,25]. Interestingly, this renal profile occurred irrespective the presence of renal functional impairment. In renal patients this renal haemodynamic profile may contribute to long term renoprotection [26].

Treatment with remikiren was associated with a gradual, significant reduction of urinary protein loss, both in overt proteinuria and in the normoalbuminuria in the essential hypertensive patients. This is likely to indicate renoprotective potential, as reduction in proteinuria has consistently been shown to
predict long-term renoprotection [27]. Despite the lack of a control group or a recovery period, the antiproteinuric effect is likely to be genuine, considering the well-controlled study conditions, with a long baseline period allowing clear-cut stabilization of proteinuria before treatment, and daily measurements of proteinuria. The antiproteinuric effect is in line with the effect of ACE inhibition and AT\(_1\)-receptor blockade [3]. However, our study design does not allow the conclusion that the antiproteinuric effect is specifically due to RAS blockade, as the lower blood pressure may have played a role.

Could there be advantages of renin inhibition as compared to other modes of RAS blockade? Theoretically it might be advantageous to block the RAS at its first and rate-limiting step. Growing evidence indicates that a substantial proportion of angII is generated from angI by non-ACE pathways, which may account for the less pronounced renal effects of ACE inhibition as compared to renin inhibition and AT\(_1\)-receptor blockade in a direct comparison [28]. It is unresolved as to whether renin inhibition might have advantages vs AT\(_1\)-receptor blockade. In our study, the fall in proteinuria with remikiren was somewhat less than that usually observed with ACE inhibition or AT\(_1\)-receptor blockade. However, we only treated for 8 days, whereas it takes approximately 4 weeks before the maximum antiproteinuric effect of ACEi and AT\(_1\) blockade is reached [29]. It would be of interest, therefore, to investigate whether more prolonged renin inhibition would further reduce proteinuria. Moreover, it might be of interest to investigate whether additive therapy with renin inhibition could provide a strategy to circumvent treatment resistance to ACE inhibition or AT\(_1\) blockade by precluding the reactive rise in renin activity with those modes of RAS blockade [30].

In conclusion, continued treatment with remikiren reduces blood pressure in hypertensive patients with normal and impaired renal function. The renal haemodynamic profile was comparable to that of ACEi and AT\(_1\)-blockade, with renal vasodilatation, an unchanged GFR, and a fall in filtration fraction. Taken together with the reduction in urinary protein loss, this suggests that specific renin inhibition exerts renoprotective effects not unlike those of ACEi and AT\(_1\)-receptor blockers. The precise mechanism of the renoprotective action of RAS blockade is still incompletely known. Whereas the clinical application of renin inhibitors has been hampered by their poor bioavailability, the data presented here suggest that renin inhibition can be a useful tool to unravel the mechanisms of the renoprotective action of RAS blockade.

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