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Effect of high dose ramipril with or without indomethacin on glomerular selectivity

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Background. Despite the accumulating evidence of their efficacy, angiotensin-converting enzyme inhibitors (ACEi) still provide imperfect renoprotection. Up-titration above conventional doses and combined therapy with other antiproteinuric agents may serve to achieve renoprotection in patients at risk of rapid disease progression.

Methods. The effect of maximum tolerated ACEi doses (ramipril 15 mg/day, range 5 to 20) alone or combined with indomethacin (75 mg × 2/day) on urinary protein excretion (UPE) and glomerular barrier size-selective function was evaluated in 19 patients with chronic non-diabetic nephropathies and persistent proteinuria.

Results. Maximum ramipril doses decreased UPE more effectively than non-ACEi therapy. Proteinuria reduction was associated with significant reduction (>50%) of the non-selective glomerular membrane shunt, but did not correlate with concomitant changes in arterial pressure and renal hemodynamics, nor was it influenced by treatment duration. The reduction in UPE and sieving coefficient of the largest neutral dextrans exceeded by twofold the reduction achieved by conventional ACEi doses in historical controls with similar renal dysfunction and proteinuria, previously studied under identical experimental conditions. Indomethacin did not influence renal effects of maximum ramipril doses and was prematurely withdrawn in six patients because of reversible side effects. Serum potassium significantly increased only in combination with indomethacin and never required treatment withdrawal.

Conclusions. Up-titration to maximally tolerated doses safely increases ACEi antiproteinuric effect and may serve to achieve maximum renoprotection in the long-term. Combination with indomethacin is poorly tolerated and ineffective. Innovative approaches are needed to use ACEi more effectively.

Heavy, persistent proteinuria is associated with unfavorable outcome in diabetic [1] and non-diabetic chronic renal disease [2]. Enhanced protein traffic through the glomerular barrier has an intrinsic renal toxicity [3] and its reduction by pharmacological manipulations that reduce protein traffic is renoprotective both in experimental [4] and human chronic nephropathies [5]. In particular, antihypertensive drugs have been used in humans to slow or even halt the progression of renal disease in diabetic [6] and non-diabetic [7, 8] proteinuric glomerulopathies. Angiotensin I-converting enzyme inhibitors (ACEi) ameliorate proteinuria and glomerular size-selective function and give more renal protection than other antihypertensive drugs for the same level of blood pressure control either in experimental animals and humans [4–8]. However, except for type 1 diabetic nephropathy, ACEi, at the doses currently employed in human nephropathies, lower proteinuria by only 20 to 50% as compared to pre-treatment [5]. This possibly explains why a substantial proportion of patients still progresses to end-stage renal disease (ESRD) despite their being on ACEi for years [5].

A goal for the future is to find the way to increase the antiproteinuric effect of ACEi further and to extend the number of patients who effectively respond to ACEi therapy [9]. Data are available that this can be safely achieved by enhancing the ACEi dose above those recommended by manufacturers to treat arterial hypertension [10]. Thus, to maximize renoprotection ACEi can be up-titrated over the doses required to normalize blood pressure (BP) using instead urinary protein as a target [5, 11, 12]. It is still unclear, however, whether the antiproteinuric effect of maximum tolerated doses of ACEi is sustained by a direct amelioration of the glomerular size-selective function and whether it can be further enhanced by other antiproteinuric drugs [13]. Recently, more proteinuria reduction has been safely achieved by combining a short-course of the non-steroidal anti-inflammatory drug (NSAID) indomethacin [14, 15] to conventional doses of an ACEi in patients with IgA nephropathy and sub-nephrotic proteinuria [16]. At this point, the question is whether further proteinuria reduction and amelioration of glomerular size-selective function can be achieved by combining a

Key words: ACE inhibition, non-diabetic nephropathies, dextran fractional clearance, glomerular size-selectivity, proteinuria, progressive renal disease.

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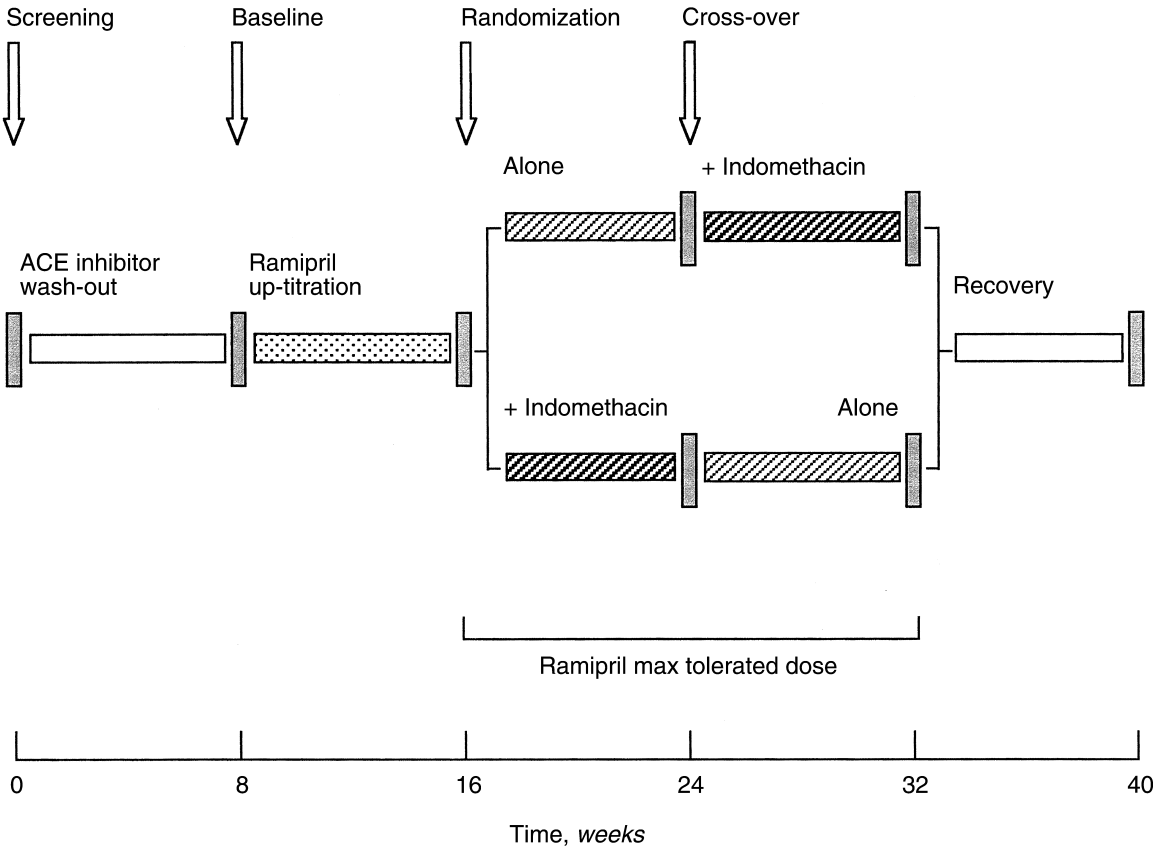

Fig. 1. Schematic representation of the study design.

prolonged course of indomethacin to an up-titrated ACEi to maximum tolerated doses. In the long term this might translate to more effective renoprotection, which may be of major clinical relevance particularly for patients who remain at an increased risk of rapid disease progression because of persistent nephrotic range proteinuria despite treatment with conventional ACEi doses.

Thus, in patients with chronic non-diabetic nephropathies and long lasting nephrotic syndrome, we evaluated whether the antiproteinuric effect of maximum tolerated ACEi doses is safely enhanced by indomethacin. Secondarily, the antiproteinuric effect of the maximum tolerated doses was compared with that of non-ACEi therapy. In parallel with the evaluation of urinary proteins, the fractional clearance of neutral dextrans of graded size were measured to evaluate whether, and to what extent, the observed changes in urinary proteins were related to concomitant changes in glomerular barrier size-selective function.

METHODS

Patients and definitions

Nineteen patients with non-diabetic chronic nephropathy and heavy persistent proteinuria were recruited from the outpatient clinic of the Unit of Nephrology, Ospedali Riuniti di Bergamo. At screening evaluation, they had persistent urinary proteins ≥3 g/24 h despite concomitant ACEi therapy for at least six months, and were without evidence of urinary tract infection or overt heart failure (New York Heart Association class III or more). Exclusion criteria were: treatment with corticosteroids, NSAIDs, or immunosuppressive drugs in the previous six months; acute myocardial infarction or cerebrovascular disease in the previous six months; severe uncontrolled hypertension (diastolic BP ≥115 mm Hg and/or systolic BP ≥220 mm Hg); evidence or suspicion of renovascular disease, obstructive uropathy, diabetes mellitus, collagen disease, cancer, abnormal serum aminotransferase concentrations, or chronic cough; drug or alcohol abuse; pregnancy, breast feeding, or ineffective contraception. Written informed consent was obtained before study entry.

Study design

Figure 1 summarizes the study design. After screening evaluation, eligible patients entered a two-month washout period from previous treatment with ACEi or angiotensin II receptor antagonists, and a one-month washout from other antihypertensives. Non-potassium sparing diuretics and occasional administration of sublingual nifed-

ipine were allowed as deemed clinically appropriate to maintain fluid balance and diastolic blood pressure <90 mm Hg. No change was introduced in the patients' previous diet, particularly in the daily protein and sodium intake. Nifedipine, however, was not allowed during urine collection in order not to affect the urinary protein excretion rate measurements. Arterial blood pressure (measured in triplicate in the sitting position after 5 minutes of rest) and 24-hour urinary protein excretion rate (mean of 3 consecutive measurements) were measured every two weeks during the entire study period. At the end of the washout period, patients underwent a renal clearance study (baseline) to evaluate glomerular filtration rate (GFR), renal plasma flow (RPF), and fractional clearance of neutral dextrans and albumin. Then, the patients entered an eight-week up-titration period, the aim of which was to identify the maximum tolerated dose of ramipril in each individual patient. To this purpose, ramipril (Hoechst Marion Roussel S.p.A, Milan, Italy) was weekly up-titrated from a starting dose of 2.5 mg/day to 5, 7.5, 10, 15, and 20 mg/day (maximum tolerated dose). One week after each increase of the study drug, blood pressure, serum creatinine and potassium values were measured. When diastolic blood pressure decreased below 70 mm Hg, the study drug was not up-titrated further to prevent the risk of symptomatic hypotension. In cases of symptomatic hypotension, serum creatinine increase by more than 30% after any dose-increase of the study drug, or hyperkalemia, the dose was decreased to the previous level. At the end of the ramipril up-titration period, the renal clearance studies were performed with the same modality of baseline evaluation. Thereafter the patients entered a crossover phase of two treatment periods in a random sequence: (*a*) two month therapy with the ACEi ramipril alone at the maximum tolerated dose; (*b*) indomethacin (75 mg twice daily) added to the maximum tolerated dose of ramipril. At the end of each treatment period of the crossover phase, the renal clearance studies were repeated. After the crossover phase, ramipril and indomethacin were withdrawn, and the patients entered a two-month recovery phase, at the end of which renal clearance studies were again performed.

Study aim

The primary aim of the study was to evaluate the changes in urinary proteins and glomerular barrier size-selectivity induced by four weeks of treatment with indomethacin added to the maximum tolerated doses of ramipril versus maximum tolerated doses alone (Study A). Secondarily, the study was aimed to compare the effects of maximum tolerated doses of ramipril with those of non-ACEi therapy (Study B).

Sample size

The primary efficacy variable of our study was the 24-hour urinary protein excretion rate. A previous study found that indomethacin added to conventional ACEi doses in 10 patients with non-nephrotic proteinuria induced a 48% reduction (from 2.3 ± 1.1 to 1.2 ± 1.1 g/24 h) in the urinary protein excretion rate [17]. Predicting—on the basis of the analyses of all patients with nephrotic range proteinuria (urinary protein excretion rate >3 g/24 h) attending our out-patient clinic—a baseline urinary protein excretion rate of 6.0 ± 2.3 g/24 h, and assuming, conservatively, as clinically relevant a 40% reduction with the combined indomethacin and ramipril therapy versus ramipril alone, it was estimated that to give this study an 80% power to detect such reductions as statistically significant ($P < 0.05$, two sided test), the predicted difference in the primary efficacy variable, at least 10 patients had to complete the study.

Clearance studies

Glomerular filtration rate and RPF were measured by inulin and paraaminohippuric acid (PAH) clearance, respectively, under a steady state of water diuresis induced by oral water loading according to a previously described procedure [17–19]. The same protocol was used for each patient during the five renal clearance studies. Briefly, after induction of diuresis, a primed infusion of inulin (Inutest; Fresenius Pharma, Graz, Austria) and PAH (Jacopo Monico, Mestre, Italy) was followed by slow intravenous administration of neutral dextran test macromolecules (130 mg/kg, Dextran-40, Rheomacrodex; Pharmacia, Uppsala, Sweden) during 10 to 15 minutes. A sustained infusion of inulin and PAH was then started to maintain constant plasma concentrations of both tracers. Approximately 15 minutes after the priming dose of inulin and PAH, the study drug was administered. After an equilibration period of about 40 minutes, three exactly-timed urine collections of 40 minutes each were made by spontaneous voiding for evaluating GFR, RPF, and albumin and neutral dextran fractional clearance (1st clearance period). Blood samples were collected at the beginning and at the end of each clearance period. Blood pressure was monitored with the patient in a supine position before solute administration and during each clearance period. Urine and plasma samples were used to determine inulin, PAH, albumin, and neutral dextran concentrations.

Inulin and PAH concentrations in plasma and urine samples were determined with colorimetric assays as previously described [17–19]. Separation of graded-size dextran molecules and inulin in plasma and urine samples was performed by gel permeation chromatography on a Sephacryl S-300 column (1.6×95 cm) using dextran standards of known molecular weight (Pharmacosmos,

Viby Sj., Denmark) for column calibration. Molecular radii of individual dextran fractions were calculated according to Oliver et al, as previously described [18–20]. Dextran concentrations in eluted fractions were determined using the anthrone reaction [21] and fractional clearance of dextran molecules was computed as:

$$\vartheta_D = (U/P)_D/(U/P)_{In} \quad [1]$$

where $(U/P)_D$ and $(U/P)_{In}$ are the urine-to-plasma concentration ratios of dextran and inulin, respectively. Mean GFR, RPF, filtration fraction (FF), and albumin fractional clearance were calculated using standard formulas as the average value of the three clearance periods, and normalized for body surface area. To take into account incomplete renal extraction of PAH, an assumed renal extraction coefficient equal to 0.7 and 0.8 was adopted for corresponding mean GFR lower or higher than 80 mL/min/1.73 m^2, respectively [22].

Other laboratory assays

Total protein concentration in 24-hour urine samples was measured by an automatic analyzer (Synchron CX5; Beckman Instruments, Fullerton, CA, USA). Other laboratory measurements [serum albumin, potassium, total cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides as well as urinary sodium and urea excretion] were done using routine laboratory techniques.

Analysis of glomerular membrane transport based on pore theory

We investigated intrinsic glomerular membrane permeability properties of macromolecules using a mathematical model of glomerular size-selectivity described in detail previously [18, 23]. This model simulates glomerular filtration of neutral test macromolecules on the basis of assumed membrane permeability properties and measured determinants of glomerular ultrafiltration. The model assumes that the glomerular membrane is perforated by cylindrical pores having a bimodal distribution of their radii. The radius of restrictive membrane pores is assumed to have a log normal probability distribution. In parallel with selective pores, a shunt pathway consisting of large pores that do not restrict the passage of largest test macromolecules is also assumed [23]. This probability distribution function of pore radii is therefore characterized by three adjustable parameters: u, s, and ω_0. The parameters u and s represent the mean and the standard deviation of the log-normal pore-size distribution, respectively, and ω_0 represents the fraction of ultrafiltrate that would pass through the shunt if plasma proteins were absent. The model is based on another freely adjustable parameter, the ultrafiltration coefficient (K_f), which is the product of hydraulic permeability and filtering surface area of the glomerular membrane. We calculated K_f (for the entire glomerular population of both kidneys) using an established model of glomerular ultrafiltration [24]. The intrinsic membrane permeability parameters were calculated as shown previously [17, 18], and the sum of squared errors between experimental and calculated sieving coefficients was minimized at single patient level during each clearance study. The approach that we used for calculating these intrinsic membrane parameters separates their effects on sieving coefficients from those owing to changes in GFR, RPF, oncotic pressure, and glomerular transmembrane hydraulic pressure difference ($\overline{\Delta P}$). This theoretical approach requires the assumption of glomerular transmembrane hydraulic pressure difference ($\overline{\Delta P}$) that cannot be directly measured in humans. In keeping with previous studies [18], we assumed $\overline{\Delta P} = 45$ mm Hg in these patients at baseline conditions, a value slightly elevated above what is believed to be a normal value (40 mm Hg), to take into consideration the moderate hypertension that characterizes our patient population. Because in experimental models of glomerular disease ACEi have been shown to selectively decrease glomerular capillary pressure [25], we assumed a value of $\overline{\Delta P} = 40$ mm Hg for theoretical analysis of sieving coefficients measured at the end of the treatment periods.

Statistical analysis

Data are expressed as mean ± SD or median and range, as specified. Data were subjected to two-way ANOVA, and significance of multiple pair-wise comparisons was determined using an unpaired t test with the Bonferroni correction [26]. Primary comparison (study A) was maximum ramipril tolerated doses alone versus maximum ramipril tolerated doses combined to indomethacin. Secondary comparisons (study B) were maximum ramipril tolerated doses versus basal and recovery.

Statistical significance level was defined as $P < 0.05$. Statistical analysis was performed using the SAS Software (Release 8; SAS Institute Inc., Cary, NC, USA).

RESULTS

Eleven male and 8 female patients, between 18 to 67 years old, were selected for study participation from February 1998 to December 1998. Eight had systolic (SBP <140 mm Hg) and diastolic blood pressure (DBP <90 mm Hg) in normal ranges without antihypertensive therapy and eleven were hypertensive. Renal biopsy had been performed in 17 patients: 6 were diagnosed with membranous nephropathy, 4 membranoproliferative nephropathy, 4 IgA nephropathy, and 3 focal segmental glomerulosclerosis.

Of the 19 patients recruited, three were withdrawn at different time points during the study because of renal cancer, myocardial infarction (during the recovery phase), and progressive worsening of renal function up to ESRD

Table 1. Demographic and clinical data at screening evaluation

Gender *M/F*	9/7
Age *years*	36 (18–67)
Systolic BP *mm Hg*	135 (110–173)
Diastolic BP *mm Hg*	82 (64–110)
Mean BP *mm Hg*	100 (81–127)
Urinary protein excretion *g/24 h*	5.5 (3.5–18.4)
Serum creatinine *mg/dL*	1.55 (0.60–3.65)

Data are mean (range).

despite stopping ramipril treatment. Main characteristics at study entry (screening evaluation) of the 16 patients who completed the study are given in Table 1. All these patients were on diuretic therapy (5 with loop diuretics, 9 with thiazides, and 2 with both). Renal function, as serum creatinine, ranged from normal to moderate renal insufficiency. The median (range 5 to 20 mg/day) dose of ramipril at the end of the up-titration phase was 15 mg/day (that is, threefold the antihypertensive dose recommended at the time the present study was designed). Specifically, seven patients reached safely the highest scheduled up-titration (20 mg/day). Up-titration was stopped in five patients before the maximum target because their diastolic blood pressure values had decreased to 70 mm Hg or less, and in two because of symptomatic hypotension. Patients who did not complete as compared to those who completed the up-titration phase had a lower mean arterial pressure value at baseline (104 ± 10 vs. 116 ± 7 mm Hg, $P = 0.034$) and during treatment with ramipril alone (90 ± 4 vs. 95 ± 6 mm Hg, $P = 0.069$) or in combination with indomethacin (94 ± 10 vs. 110 ± 9 mm Hg, $P = 0.006$). These differences were not associated with differences in urinary sodium excretion. During the following two treatment periods with maximum ramipril doses alone or combined to indomethacin, each patient was maintained on the dose of ramipril achieved at the end of the up-titration period.

Study A. Comparative analyses between treatment with ramipril alone or combined to indomethacin

Efficacy assessment. Proteinuria, SBP, DBP, mean arterial pressure (MAP), renal hemodynamic parameters, albumin fractional clearance, and the sieving profile of dextran molecules, measured at the end of the treatment period with maximum tolerated dose of ramipril alone or combined to indomethacin were comparable (Tables 2 to 4 and Fig. 2). The above parameters, with the exception of GFR, also were comparable at the end of the treatment period with ramipril maximum tolerated doses and of the up-titration period.

Safety assessment. With maximum tolerated doses of ramipril alone or combined with indomethacin, the mean serum potassium was significantly higher than at baseline, but none of the patients had to be withdrawn from the study because of refractory hyperkalemia. Mild hyperkalemia (serum potassium between 5.5 and 6 mEq/L) occurred only in 2 out of 16 patients during the combined treatment with ramipril and indomethacin (Table 5) No major change in serum lipid profile was reported during the study phases, although a significantly lower mean serum total cholesterol value was observed at the end of the combined ramipril plus indomethacin treatment than in the ramipril titration and recovery phases (Table 5). Changes in GFR were significantly correlated with urinary sodium excretion during the combined treatment with ramipril and indomethacin ($r = 0.61$, $P = 0.01$), but not during treatment with ramipril alone ($r = 0.43$, $P = 0.11$). Indomethacin had to be withdrawn before completion of the two-month combined treatment with ramipril in six patients because of dyspepsia refractory to symptomatic treatment ($N = 2$), edema ($N = 2$), somnolence ($N = 1$), and serum creatinine increase >30% versus baseline ($N = 1$). The adverse events fully reversed and all patients recovered after indomethacin withdrawal.

Study B. Comparative analyses versus baseline

Clinical parameters. Twenty-four hour proteinuria at the end of the maximum tolerated ramipril dose period significantly decreased as compared to baseline (mean reduction 29%) and fully recovered after ramipril withdrawal (Table 2). Although differences were not statistically significant, proteinuria reduction tended to be greater (45 to 50%) in patients treated with furosemide alone or in combination with thiazides, than in those treated with thiazides alone (about 20%). SBP, DBP, and MAP followed a similar trend, with only a partial recovery of SBP after ramipril withdrawal (Table 3). Neither changes in proteinuria and blood pressure were significantly associated with urinary sodium excretion (data not shown). The GFR significantly decreased with maximum tolerated ramipril dose and only partially recovered after ramipril withdrawal (Table 4). At variance, RPF and filtration fraction (FF) did not change significantly throughout the three study periods (Table 4). Albeit the change did not achieve statistical significance, mean albumin fractional clearance numerically decreased with the maximum tolerated ramipril dose and fully recovered after ramipril withdrawal (Table 4). Urinary sodium, potassium, and urea excretion did not change significantly through out the three considered periods (Table 2).

Neutral dextran fractional clearance. Sieving coefficients of neutral dextran molecules of graded sizes (20 to 70 Å) measured in the basal condition, at the end of the maximum tolerated ramipril dose, and after the recovery period are reported in Figure 3. Fractional clearances of small dextran molecules (radii <36 Å) were not significantly affected by maximum tolerated dose of ramipril as compared to baseline. Sieving coefficients of larger dextran molecules (radii 36 to 70 Å) were sig-

Table 2. Urinary protein, sodium, potassium, and urea excretion at baseline, at the end of ramipril titration phase (titration), at the maximum-tolerated ramipril dose (MR), at the indomethacin plus maximum-tolerated ramipril dose (MR+Ind), and after recovery

	Baseline	Titration	MR	MR+Ind	Recovery
Protein excretion rate *g/24 h*	5.6 ± 1.6	4.0 ± 1.4[ab]	4.0 ± 2.2[ab]	3.9 ± 2.1[ac]	5.1 ± 1.9
Sodium excretion *mEq/24 h*	199 ± 93	172 ± 82	195 ± 107	184 ± 83	181 ± 90
Potassium excretion *mEq/24 h*	64.3 ± 17.1	61.3 ± 21.0[b]	66.2 ± 27.5	65.7 ± 22.9	73.6 ± 21.6
Urea excretion *g/24 h*	22.5 ± 7.4	19.0 ± 5.9	19.5 ± 7.5	21.8 ± 6.5	20.7 ± 6.9

Data are mean ± SD.
[a] $P < 0.01$ vs. baseline
[b] $P < 0.05$ and [c] $P < 0.01$ vs. recovery

Table 3. Systolic, diastolic and mean blood pressure measured at baseline, at the end of ramipril titration phase (titration), at the maximum-tolerated ramipril dose (MR), at the indomethacin plus maximum-tolerated ramipril dose (MR+Ind), and after recovery

	Baseline	Titration	MR	MR+Ind	Recovery
SBP *mm Hg*	142 ± 16	129 ± 14[b]	128 ± 13[b]	130 ± 14[a]	133 ± 11[a]
DBP *mm Hg*	89 ± 11	78 ± 12[bc]	76 ± 9[bd]	81 ± 11[b]	85 ± 9
MAP *mm Hg*	107 ± 12	95 ± 12[bc]	93 ± 10[bc]	97 ± 12[b]	101 ± 9[a]

Data are mean ± SD. Abbreviations are: SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure.
[a] $P < 0.05$ and [b] $P < 0.01$ vs. baseline
[c] $P < 0.05$ and [d] $P < 0.01$ vs. recovery

nificantly decreased with maximum tolerated ramipril doses as compared to baseline, and almost completely recovered after ramipril withdrawal (radii 64 to 70 Å).

Theoretical analysis of glomerular size-selective function. Dextran sieving coefficients and renal hemodynamic parameters were used as input data for the theoretical analysis of glomerular size-selective function in the different phases of the study. The results of the theoretical analysis are reported in Table 6. While the change did not achieve statistical significance, the calculated values of K_f, mean pore radius (u) and the spreading (s) of the pore-distribution numerically increased with maximum tolerated ramipril dose as compared to baseline values, and fully recovered after ramipril withdrawal (Fig. 4).We also calculated the shunt parameter ω_0 that was reduced by more than 59% by maximum tolerated ramipril dose as compared to baseline and partially recovered after ramipril withdrawal (29%; Table 6 and Fig. 4).

DISCUSSION

The results of the present study show that in patients with non-diabetic chronic nephropathies and long-lasting, persistent nephrotic proteinuria, indomethacin added to maximum tolerated doses (up to 20 mg/day, on average 15 mg/day) of ramipril, does not further ameliorate proteinuria and glomerular size-selectivity and is poorly tolerated. However, maximum tolerated doses of ramipril ameliorated both proteinuria and size selective function as compared to non-ACEi therapy, even in patients with unrestricted sodium intake. These changes did not correlate with concomitant reduction in arterial blood pressure or with any concomitant change in renal hemodynamics, nor were they influenced by treatment duration. However, although differences were not statistically significant, proteinuria reduction was almost double in patients treated with loop diuretics than in those treated with thiazides.

Previous studies found a further reduction in urinary proteins when other antiproteinuric drugs, such as NSAIDs, mostly indomethacin [16], were given in combination with conventional ACEi doses. Indomethacin effectively reduces proteinuria in experimental animals [27] and in nephrotic patients [14, 15], and has complementary effects to ACEi on membrane permeability [28] and contrasting effects on glomerular hemodynamics [29]. Here, we found no further reduction in urinary protein excretion nor in the glomerular sieving coefficients of small and large neutral dextran macromolecules when patients were receiving the combined therapy for eight weeks, as compared to ramipril alone at maximum dose. This contrasts with previous findings that the addition of indomethacin to an ACEi at a conventional dose regimen offered additive antiproteinuric effect in patients with renal disease [16]. Differences in treatment duration of indomethacin and in dose regimen of ACEi that are not therapeutically equivalent, as for the antiproteinuric effect, may account for different outcomes on proteinuria of the combined therapy observed in the present study. Conceivably, a longer treatment period might have been more effective. However, eight weeks is a relatively standard period that is sufficient to achieve the maximum antiproteinuric effects with other drugs such as ACE inhibitors or angiotensin II receptor antagonists. Moreover, without a substantial effect on urinary proteins, a prolonged treatment period might offer only marginal benefits that might be overwhelmed by the potential risks of more nephrotoxicity. Regardless of the involved mechanisms, besides being ineffective as antiproteinuric agent, indomethacin was not well tolerated and tended to decrease the GFR in patients with less sodium intake, which adds a further safety concern on the validity of combined therapy in patients with proteinuric

Table 4. Kidney functional parameters at baseline, at the end of ramipril titration phase (titration), at the maximum-tolerated ramipril dose (MR), at the indomethacin plus maximum-tolerated ramipril dose (MR+Ind), and after recovery

	Baseline	Titration	MR	MR+Ind	Recovery
GFR *mL/min/1.73 m²*	56 ± 31	51 ± 23	45 ± 25[ab]	50 ± 28	48 ± 23[a]
RPF *mL/min/1.73 m²*	442 ± 280	464 ± 241[c]	392 ± 209[b]	435 ± 269	373 ± 220
FF %	14 ± 7	12 ± 4[c]	12 ± 4	12 ± 4	14 ± 6
Albumin FC $\times 10^{-3}$	2.49 ± 1.83	1.72 ± 1.65	1.69 ± 1.09	1.86 ± 1.61	2.43 ± 1.73

Data are mean ± SD. Abbreviations are: GFR, glomerular filtration rate; RPF, renal plasma flow; FF, filtration fraction; FC, fractional clearance.
[a] $P < 0.05$ vs. baseline
[b] $P < 0.05$ vs. titration
[c] $P < 0.05$ vs. recovery

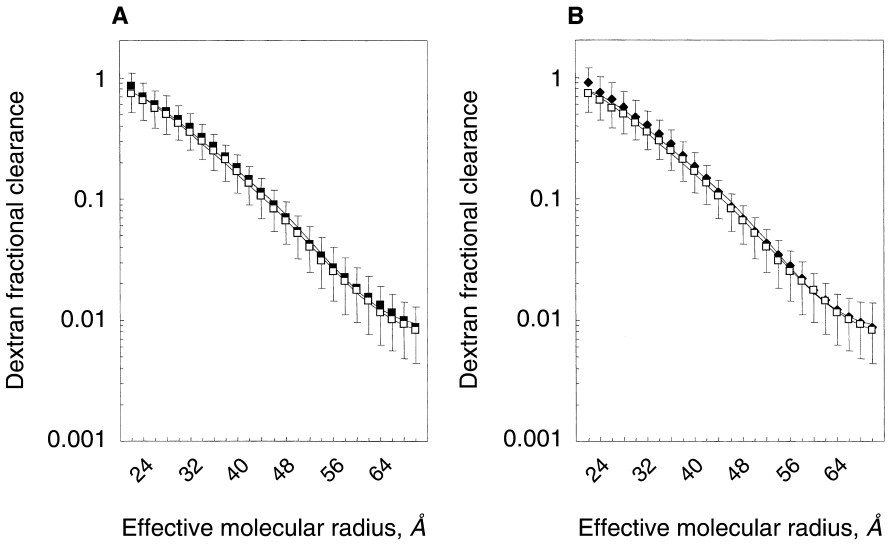

Fig. 2. (*A* and *B*) Fractional clearance of neutral dextran macromolecules as a function of effective molecular radius measured at the end of the ramipril titration phase (titration; ■), at the maximum-tolerated ramipril dose phase (MR; □), and of the indomethacin plus maximum-tolerated ramipril dose phase (Ind+MR; ◆). The continuous line represents calculated fractional clearance according to the log normal + shunt model and optimal parameter values.

Table 5. Serum potassium, total and high-density lipoprotein (HDL) cholesterol, and triglycerides at baseline, at the end of the ramipril titration phase (titration) at the maximum-tolerated ramipril dose (MR), at the indomethacin plus maximum-tolerated ramipril dose (MR+Ind), and after recovery

	Baseline	Titration	MR	MR+Ind	Recovery
Serum potassium *mEq/L*	4.2 ± 0.5	4.4 ± 0.6	4.5 ± 0.4	4.8 ± 0.5[abc]	4.4 ± 0.4
Serum total cholesterol *mg/dL*	277 ± 96	291 ± 108[c]	279 ± 101	267 ± 112[bc]	313 ± 134
Serum HDL cholesterol *mg/dL*	53.4 ± 14.5	55.3 ± 18.8	51.9 ± 17.2	52.4 ± 14.6	56.3 ± 13.8
Serum triglycerides *mg/dL*	188 ± 99	183 ± 137	199 ± 137	178 ± 124	213 ± 142

Data are mean ± SD.
[a] $P < 0.01$ vs. baseline
[b] $P < 0.05$ vs. titration
[c] $P < 0.05$ vs. recovery

chronic nephropathies already on maximum tolerated ACEi doses. Moreover, the blood pressure tended to be higher during combined ramipril-indomethacin therapy than during therapy with ramipril alone. This might have offset, at least in theory, some of the antiproteinuric effects of indomethacin.

On the other hand, theoretical analysis of dextran fractional clearance values showed that maximum tolerated ramipril doses, as compared to non-ACEi therapy, had little effect on the mean (u) and the distribution (s) of membrane pore-radii, but reduced by more than 50% the relative importance of the non-selective shunt pathway, as indicated by mean change in calculated shunt parameter (ω_0; Fig. 4) which is responsible for the passage of circulating macromolecules into the urinary space. Thus, maximum ACE inhibition decreased urinary protein excretion by ameliorating the glomerular size-selective function.

In addition, maximum tolerated ramipril doses allowed a remarkable reduction (27%) in proteinuria to be achieved versus the screening evaluation, when patients were on chronic treatment with conventional ACEi doses.

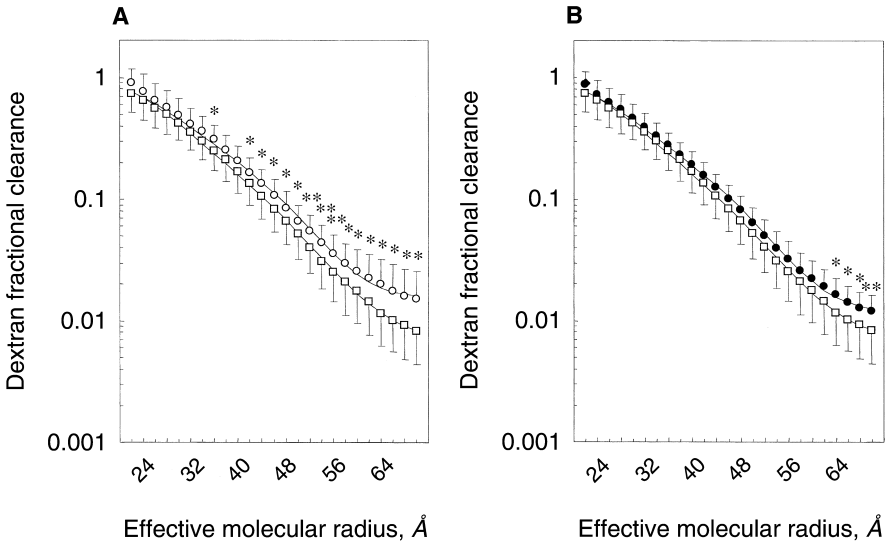

Fig. 3. (*A* and *B*) Fractional clearance of neutral dextran macromolecules as a function of effective molecular radius measured at baseline (○), at the end of the maximum-tolerated ramipril dose (MR; □), and after recovery (●). The continuous line represents calculated fractional clearance according to the log normal + shunt model and optimal parameter values. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. MR.

Table 6. Assumed and calculated glomerular membrane permeability parameters at baseline, at the end of ramipril titration phase (Titration), at the maximum-tolerated ramipril dose (MR), at the indomethacin plus maximum-tolerated ramipril dose (MR+Ind), and after recovery

	Baseline	Titration	MR	MR+Ind	Recovery
$\overline{\Delta P}$ *mm Hg*	45	40	40	40	45
K_f *mL/min/mm Hg*	2.18 ± 1.37	2.51 ± 1.52	2.44 ± 2.32	2.62 ± 1.69	1.89 ± 1.16
u Å	54.3 ± 11.2	53.0 ± 8.1	50.6 ± 8.1	54.0 ± 7.1	54.9 ± 8.1
s Å	1.16 ± 0.11	1.16 ± 0.07	1.19 ± 0.07	1.15 ± 0.06	1.15 ± 0.08
ω_0 $\times 10^{-3}$	13.0 ± 10.4	6.8 ± 5.3	5.3 ± 3.7[a]	5.8 ± 3.1	9.2 ± 3.9[b]

Data are mean ± SD. Abbreviations are: ΔP, mean transmembrane pressure difference; K_f, ultrafiltration coefficient; *u*, mean pore size; *s*, standard deviation of corresponding normal probability distribution; ω_0, shunt parameter.

[a] $P < 0.05$ vs. Baseline

[b] $P < 0.01$ vs. MR

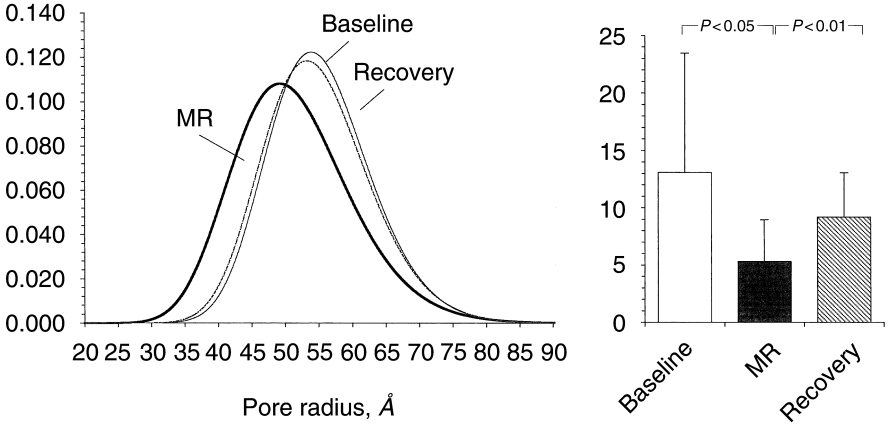

Fig. 4. Log-normal probability distribution of glomerular membrane pore-size and mean group shunt parameter (ω_0) at baseline, at the end of the maximum-tolerated ramipril dose phase (MR), and after recovery.

However, since neutral dextran clearances were not measured at screening evaluations, the present study could not demonstrate that this effect was sustained by a further amelioration of glomerular size-selective function. To address this issue, we compared the effect of maximum ACEi with that of conventional doses evaluated in a previous series of patients given ACEi for a similar period of time (10 weeks) with similar mean (range) serum creatinine concentration [1.3 (1.1 to 3.9) mg/dL] and proteinuria [5.6 (0.7 to 13.3) g/24 h] at study inclusion, and evaluated under the same experimental conditions (that is, by using the same clearance procedures and

analytical methods to measure changes in proteinuria, GFR, RPF and glomerular barrier permeability parameters) [19]. These comparative analyses showed that, despite an identical reduction (−11.5%) in mean arterial pressure, the maximum tolerated as compared to conventional antihypertensive doses allowed a more effective reduction in proteinuria to be achieved (27.6 vs. 10.5%), which was associated with a reduction in the fractional clearance of largest dextran molecules that exceeded by two- to threefold the reduction achieved by conventional doses [19]. This effect was not accompanied by detectable differences in systemic blood pressure and GFR, and is most likely dependent on enhanced inhibition of locally formed angiotensin II that, in line with experimental observations, are responsible for rearrangement of junctional complex of podocyte filtration slits [30]. Altogether these findings may have major clinical implications, since the extent of short-term proteinuria reduction correlates with subsequent GFR decline in the long-term [7, 31]. Up-titrating ACEi therapy to achieve the maximum possible reduction in proteinuria therefore may be the way to go in the next future to have maximum renoprotection in the long-term [9, 32].

Despite the effect on urinary proteins, up-titrating ACEi can be difficult in normotensive or mild hypertensive patients, who may be more susceptible to symptomatic hypotension, or in those with more advanced renal disease and/or diabetes who may be more prone to hyperkalemia. However, only two patients in our study experienced symptomatic hypotension. These two patients already had relatively lower blood pressures at study entry. Two other patients had a mild hyperkalemia during combined ramipril and indomethacin therapy that was easily controlled by correction of metabolic acidosis and did not require treatment down-titration or withdrawal. Tolerability to ACEi therapy may vary from patient to patient and the antiproteinuric regimen must be empirically titrated in each individual patient according to efficacy and tolerability [9, 32].

In summary, in patients with chronic non-diabetic nephropathy and nephrotic range proteinuria, ACEi at maximum tolerated doses that are well above the current recommendation for an ideal blood pressure control, despite having no additional effect on arterial blood pressure, reduced proteinuria and significantly ameliorated glomerular size-selectivity without side effects. Amelioration of proteinuria and glomerular size-selective function was superior to that previously observed with conventional ACEi doses and occurred even without a restricted sodium intake. Indomethacin did not ameliorate the glomerular permselective function further, but rather raised tolerability and safety concerns.

We conclude that in order to enhance their renoprotective properties, ACEi can be used at doses higher than current clinical practice. Proteinuria reduction rather than blood pressure is probably the right target. Nephrologists should now abandon indomethacin when ACEi are used properly.

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