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Effects of combined ACE inhibitor and angiotensin II antagonist treatment in human chronic nephropathies

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Background. Proteinuria predicts renal disease progression, and its reduction by angiotensin-converting enzyme inhibitors (ACEi) or angiotensin II receptor antagonists (ARA) is renoprotective.

Methods. In this prospective, randomized, cross-over study of 24 patients with nondiabetic, chronic nephropathies, we compared the effects on proteinuria, renal hemodynamics, and glomerular permselectivity of 8 weeks with comparable blood pressure control achieved by benazepril (10 mg/day) and valsartan (80 mg/day) combined therapy with those achieved by benazepril (20 mg/day) or valsartan (160 mg/day) alone.

Results. Despite comparable changes in blood pressure and glomerular filtration rate (GFR), combined therapy decreased proteinuria more than benazepril (−56% vs. −45.9%, $P = 0.02$) and valsartan (−41.5%, $P = 0.002$). Changes in urinary protein to creatinine ratio followed the same trend. Filtration fraction and renal vascular resistances (RVR) decreased more with combined (−14.7%, −23.7%) or benazepril (−12.4%, −20.5%) than with valsartan (−2.7%, −12.5%, $P < 0.05$ vs. both). RVR changes, adjusted for GFR changes, were associated with those in proteinuria ($P < 0.05$). Changes in glomerular permeability were comparable and did not predict different changes in proteinuria in the three groups.

Conclusion. At comparable blood pressure, combined ACEi and ARA decreased proteinuria better than ACEi and ARA. The greater antiproteinuric effect most likely depended on an ACEi-related hemodynamic effect, in addition to glomerular size selectivity amelioration. Long-term combined ACEi and ARA therapy may be more renoprotective than treatment with each agent alone.

Key words: proteinuria, ACE inhibitors, angiotensin II receptor antagonists, glomerular permeability, progressive nephropathies.

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Proteinuria is a major predictor of decline in renal function in patients with non-diabetic, proteinuric renal disease. The Modification of Renal Disease (MDRD) [1] and Ramipril Efficacy in Nephropathy (REIN) [2] trials found that patients with a greater degree of proteinuria had a more rapid decline in glomerular filtration rate (GFR) and a faster progression to end-stage renal disease (ESRD). The REIN trial demonstrated that an initial response to angiotensin-converting enzyme inhibitor (ACEi) therapy, manifested by a decrease in proteinuria over the first month of treatment, correlated with a slowing of the rate of loss of GFR and of progression to ESRD over the following 2 years [2]. The GFR in a subset of patients in this trial stabilized and even improved over the 3 years of continued ramipril therapy. For each of these patients, the change in GFR slope while on ACEi was paralleled by a further reduction in 24-hour protein excretion [3]. This evidence suggests that decreasing proteinuria, regardless of its cause, is beneficial in slowing progressive loss of GFR and in reducing the risk of terminal renal failure.

The best way to reduce proteinuria, however, is unclear. An ACEi has unique antiproteinuric effects not found in other antihypertensive drugs. Blockade of the renin-angiotensin system (RAS) with ACEi results in a decrease in the glomerular hyperfiltration that follows nephron loss [4] and in an improvement of glomerular sieving properties that may be independent of their hemodynamic effects [5]. ACEi has been shown in multiple animal models [4] and in long-term clinical studies to reduce proteinuria and slow GFR decline in diabetic and nondiabetic renal disease [2, 6]. Angiotensin II receptor antagonists (ARA), which block the angiotensin II type 1 receptor (AT-R1) but not the type 2 receptor (AT-R2), appear to be equally efficacious as ACEi [7]; however, there are no long-term studies that directly compare the efficacy of ACEi to

ARA. The combination of an ARA and ACEi has been suggested as a way to maximize RAS blockade by affecting both the bioavailability of angiotensin II through ACEi and also by affecting its activity at the receptor level. An ACEi has the additional properties of blocking the breakdown of bradykinin, a vasodilator that also stimulates nitric oxide production [4]. ARA do not affect the activity of the AT-R2, which appears to be important in vasodilation [4]. The combination of these two drugs may be a way to block the effects of angiotensin II at the AT-R1 level, while achieving both increased bradykinin levels and activation of the AT-R2.

Experimental [abstract; Zoja C et al, *J Am Soc Nephrol* 6:1101, 1995] and human data [8, 9] are available that ACEi and ARA in combination more effectively reduce proteinuria than the two agents alone. However, it is unknown if the combination of these two classes of drugs is more effective because of a synergistic effect on glomerular barrier size selectivity or rather because of a hemodynamic effect [4, 8–10].

The primary aim of the study was to test the hypothesis that in patients with proteinuric chronic nephropathies, combined therapy with half doses of ACEi and ARA may achieve greater reduction of proteinuria than treatment with full doses of each drug alone. Half doses were used to achieve comparable blood pressure control in the combined and single agent treatment groups. Second, the study was intended to assess to which extent the antiproteinuric effect of each treatment was due to an effect on glomerular barrier size selectivity or rather on a specific intrarenal hemodynamic effect.

METHODS

Subjects

The study population was drawn from nondiabetic, hypertensive patients with chronic renal disease over the age of 18 years attending the Outpatient Clinics of the Unit of Nephrology of the Ospedali Riuniti of Bergamo or of the Clinical Research Center "Aldo & Cele Daccò" of the Mario Negri Institute of Bergamo. Inclusion criteria were a creatinine clearance between 20 and 70 mL/min/1.73 m^2 body surface area (BSA) and urinary protein excretion rate $\geq$1 g/24 hours (average of two measurements in two urine collections two weeks apart). Hypertension was defined as sitting diastolic blood pressure between 90 and 115 mm Hg (or less in patients with concomitant antihypertensive therapy). Patients were excluded if they had a specific contraindication to temporarily withdrawal of chronic treatment with ACEi or ARA, or received treatment with steroids, nonsteroidal antiinflammatory drugs (NSAIDs), immunomodulators, or cytostatic agents in the last 6 months, had renal vascular disease, obstructive uropathy, unstable angina, had suffered an acute myocardial infarction or cerebral vascular accident in the last 6 months, were NYHA class III-IV, had serum potassium of >6 mEq/L, despite control of metabolic acidosis, clinically significant hepatic disease [serum glutamic-oxalacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), serum alkaline phosphatase >3 times of the upper normal limit, or serum bilirubin >1.5 mg/dL], white blood cell count <3000/mm^3, clinical suspicion of renal vein thrombosis, known hypersensitivity to ACEi or ARA, cancer, collagen vascular disease, treatment with other investigational drugs, were pregnant or lactating, were not using effective contraception, or were unwilling to consent to participation. All patients gave informed consent prior to enrolling in the study.

Study protocol

This was a single-center, prospective, randomized, open-label crossover study comparing the effects of treatment with either valsartan (A) alone, benazepril (B) or the combination of valsartan and benazepril (C) in three randomized periods of 8 weeks each. The study protocol was approved by the Ethics Committee of the Clinical Research Center for Rare Diseases "Aldo e Cele Daccò" of the Mario Negri Institute of Bergamo.

After obtaining informed consent, the subjects entered an 8-week run-in period during which any previously used ACEi, ARA, and potassium-sparing diuretics were stopped. Subjects were allowed to continue loop or thiazide diuretics and antihypertensive agents (clonidine) that did not affect glomerular barrier size selectivity to maintain a diastolic blood pressure of less than 90 mm Hg.

At the end of the run in period, the patients were randomized to one of six treatment sequences: ABC, BCA, CAB, CBA, ACB, or BAC (Fig. 1). Each treatment was given orally at 8:00 a.m. daily for 8 weeks. During each period, the study drug was started at dose level 1 (10 mg benazepril, 80 mg valsartan, or 5 mg benazepril and 40 mg valsartan) and then increased to dose level 2 after 2 weeks (20 mg benazepril, 160 mg valsartan, or 10 mg benazepril and 80 mg valsartan). Seven days after the start of dose level 2, blood pressure, serum creatinine, and potassium were measured. If hyperkalemia or symptomatic hypotension occurred, the dose level was decreased to level 1 or the study treatment was withdrawn.

The target blood pressure during the study was a diastolic pressure of 90 mm Hg or less. Adjuvant antihypertensive (clonidine or loop or thiazide-type diuretics) agents that do not interfere with the glomerular barrier size selectivity were used as needed. Diuretics were held for 24 hours prior to and for 48 hours following the start of a new study drug. Patients were advised not to modify their usual protein and sodium intake throughout the whole study period. At the end of the run-in period (baseline) and at the end of each of the treatment peri-

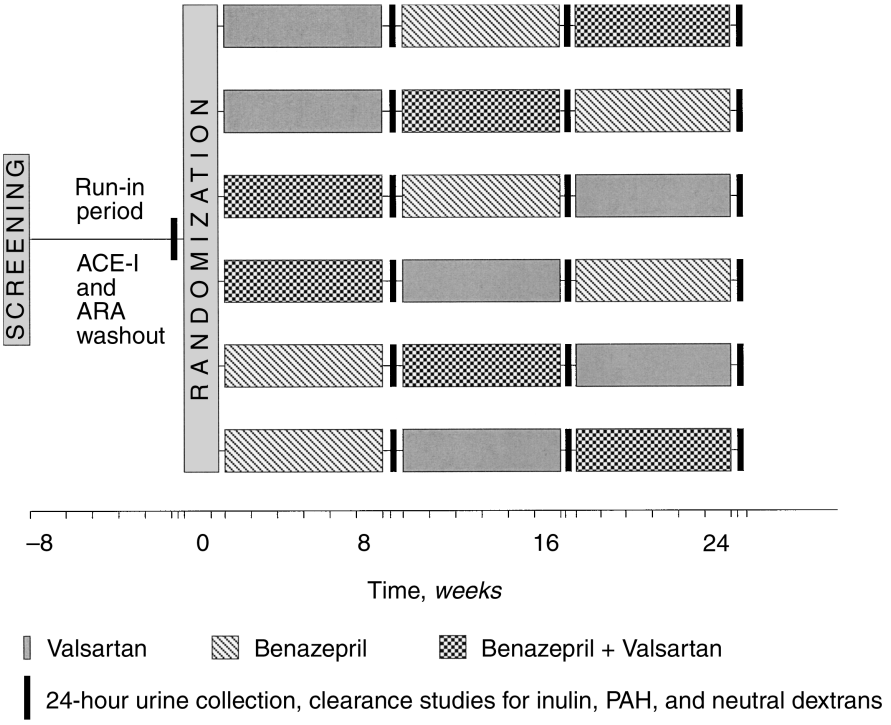

Fig. 1. Study design. Patients were randomized to six treatment sequences. Each sequence included four patients. Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ARA, angiotensin receptor antagonist.

ods, three 24-hour collections of urine were obtained for creatinine clearance, sodium, and protein measurements.

Measurements

Prior to the clearance studies, three blood pressure measurements were taken by automated cuff on the dominant arm 2 minutes apart with the patient in the sitting position and after 5 minutes of rest. Systolic and diastolic blood pressures were the average of the three readings. Mean arterial pressure was calculated using the standard formula from the average of the three readings. Blood chemistries were measured by standard laboratory techniques from blood samples taken prior to the clearance studies. Twenty-four–hour urine protein measurements were averaged from three 24-hour urine collections made during the 3 days prior to the clearance studies. Creatinine clearance and ratio of urinary protein to urinary creatinine were measured from the last 24-hour collection of urine.

Clearance studies

Each patient underwent inulin and para-aminohippuric acid (PAH) clearance studies to determine GFR and effective renal plasma flow (ERPF), respectively, after the washout period (referred to as baseline) and at the end of each treatment period. Filtration fraction (FF) and renal vascular resistances (RVR) were calculated by standard formulas.

As previously described [10, 11] the patients were admitted to the metabolic unit the morning of the clearance studies after an overnight fast. After induction of diuresis, a priming infusion of inulin and PAH was delivered followed by a sustained infusion. A slow infusion of 130 mg/kg dextran 40 was given following the loading dose of inulin and PAH. After an equilibrium period of approximately 40 minutes, three exactly timed urine samples 40 minutes apart were made by spontaneous voiding and were collected. Blood samples were collected at the beginning and end of each clearance period. Urine and plasma samples obtained during the first period were used to determine the fractional clearance of dextran molecules of graded size (effective molecular radius ranging from 20 to 70 Å).

Analytic methods

Inulin and PAH concentrations in plasma and urine samples were determined using previously described colorimetric assays [11, 12]. GFR was calculated as the average of the three inulin clearances. ERPF was calculated from average PAH clearance assuming a renal extraction coefficient of 0.7 for patients with a GFR $\leq$80 mL/min/1.73 m^2 and of 0.8 for patients with a GFR >80 mL/min/1.73 m^2 [13]. 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 (1.65 $\times$ 95 cm) (Amersham Biosciences, Ltd., Buckinghamshire, UK). Column calibration was performed using dextran standards of known molecular weight (Pharmacosmos, Viby Sj, Denmark). The molecular radius of individual dextran fractions was calculated ac-

cording to direct measurements of neutral dextran molecules as previously reported [14] that relate effective molecular radii and mean molecular weight of neutral dextran. Urine to plasma concentration ratio for inulin was calculated from the inulin concentrations in eluted fractions. Fractional clearance of dextran macromolecules was computed using:

$$\Theta_D = (U/P)_D / (U/P)_{IN}$$

where $(U/P)_D$ and $(U/P)_{IN}$ are the urine to plasma concentration ratios of dextran and inulin, respectively. RVR and FF were calculated using the standard formulas.

Theoretical analysis of glomerular membrane transport

We investigated intrinsic glomerular membrane permeability properties to macromolecules using the mathematical model of glomerular size selectivity described in detail previously [12, 15, 16]. This model allows separation of the effects on membrane permeability changes from the effects of glomerular hemodynamic alterations on glomerular filtration of neutral test macromolecules. 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 macromolecules is also assumed [12, 15]. This distribution of pore radii is therefore characterized by three adjustable parameters: u, s, and ω_o. The parameters u and s represent the mean and the standard deviation of the corresponding normal probabilities distribution, while ω_o represents the fraction of the ultrafiltrate that would pass through the shunt if plasma proteins were absent [12, 15]. The model is based on another freely adjustable parameter, the ultrafiltration coefficient (K_f), the product of hydraulic permeability and filtering surface area of the glomerular membrane. We calculated K_f (extended to the entire glomerular population in both kidneys) using an established model of glomerular ultrafiltration [17]. The intrinsic permeability parameters were calculated, as shown previously [11, 12], minimizing the sum of squared errors between experimental and calculated sieving coefficients, at single patient level, during each clearance study.

Sample size

The sample size was calculated by nQuery Advisor, release 3.0 (Statistical Solutions, Ltd., Cork, Ireland). The primary efficacy variable of the study was the percent reduction (versus baseline) in 24-hour urinary protein excretion rate. On the basis of all patients with chronic, nondiabetic nephropathies and proteinuria $\geq$1 g/24 hours attending our outpatient clinic, a baseline 24-hour urinary protein excretion rate of 3.5 $\pm$ 2.0 g/24 hours was predicted. Assuming, on the basis of previous studies, a 40% reduction with valsartan or benazepril therapy, and considering as clinically relevant a further 20% reduction with combined therapy, we predicted a decrease in urinary proteins from 3.5 to 2.1 g/24 hours with single benazepril or valsartan therapy and from 3.5 to 1.4 g/24 hours with combined therapy (i.e., from 3.5 to 1.4 g/24 hours). Thus, with the assumption of a mean $\pm$ standard deviation (SD) difference in urinary protein excretion reduction between combined and single drug treatments of 0.7 $\pm$ 1.0 g/24 hours, it was estimated that to give the study an 80% power to detect such difference as statistically significant ($P < 0.05$), 19 patients had to complete the study.

Statistical analysis

The study was a three-period, three-treatment crossover study. The possibility that a "period effect" (i.e., a time-dependent trend that can affect the experiment as a whole, regardless of the treatment under evaluation) or a "carryover" effect (i.e., the persistence of the effect of a treatment applied in one period in a subsequent period of treatment) could affect the study findings was addressed both "a priori" when the study was designed and "a posteriori," before performing the final analyses.

To "a priori" prevent or limit the possibility of a "period effect," we introduced a degree of balance into the study design, with a scheme of randomization allowing that every treatment is represented in every period with the same frequency. Overall, we had six different sequences with the three treatments valsatran (A), benazepril (B), and their combination (C): ABC, BCA, CAB, CBA, ACB, and BAC. An equal number of patients (N = 4) per sequence was randomized. Since no patient was prematurely withdrawn during the study completion, at study end this balance was fully respected.

To prevent or limit the risk of a "carryover" effect, we planned treatment periods of 8 weeks. Actually, previous studies (including some from our group) found that the effects of ACEi and ARA on urinary proteins and glomerular permselectivity are fully reversible within 4 weeks [7, 11, 12]. Thus, prolonging each treatment period for 8 weeks allowed ruling out any residual effect of previous treatment at week 8, when proteinuria and size selectivity were measured.

To verify that the above design allowed ruling out a significant "period" and "carryover" effect, we used the approach suggested by Senn [18]. Specifically, an "a posteriori" analysis of variance using SAS General Linear Models (GLM) procedure (SAS Institute, Inc., Cary, NC, USA), creating a data set with 72 outcomes (three each for 24 patients) was carried out. The following variables were created: patients, period, and treat, which recorded each of the 72 outcomes for which patient it was recorded, in which period, and which treatment the

Table 1. Patient characteristics

Male/female	23/1
Mean age *years*	48.9 ± 13.2
Diagnosis	
IgA nephritis	11
Chronic glomerulonephritis (nondiagnostic)	4
Other	4
No biopsy	5

patient was receiving. Patient category was a variable of 24 levels, while period and treat were three each. Subsequently, it also defined a factor "carry" with levels A, B, or C for the previous treatment and level Z when there was no previous treatment. In absence of a statistically significant "period" and "carryover" effect, a matched-pairs *t* approach was used. Percent changes were evaluated by means of one-group *t* test. Relationships between variables were analyzed by repeated measures of ANOVA mixed models.

Treatment comparisons of combined therapy versus valsartan alone and combined therapy versus benazepril alone represented the two main contrasts of interest. To adjust for this multiplicity of the two contrasts and to achieve an overall significance level of $P < 0.05$, each contrast was analyzed at a two-sided significance level of 0.025 (Bonferroni adjustment). No formal comparisons were performed between baseline evaluation and each treatment period and between benazepril and valsartan alone.

The skewed distribution of 24-hour urinary protein excretion, urinary protein/urinary creatinine ratio (P/C), albumin, and neutral dextran fractional clearances were normalized by natural logarithmic transformation prior to statistical analyses. All evaluations were done with SAS software (release 8.0). Data were expressed as mean + SD, or standard error (SE), as specified in the text, or absolute and percent frequencies.

RESULTS

Twenty-four subjects were enrolled and all 24 completed the trial. Twenty-three males and one female were enrolled in the study. All patients were Caucasian. The average age of the patients was 48.9 ± 13.2 years. Clinical characteristics are listed in Table 1. Twenty-three of the 24 patients achieved the target level 2 dose of each medication. The remaining patient finished the trial while on the level 1 dose. That patient did not advance to the level 2 dose because the diastolic blood pressure was consistently below 90 mm Hg on the level 1 dose. Before study analyses were performed, no statistically significant "period" ($P = 0.12$) and "carryover" ($P = 0.86$) effect was found.

Clinical and laboratory parameters

Despite comparable changes in systolic and diastolic arterial blood pressure and creatinine clearance, combined treatment with half doses of benazepril and valsartan decreased 24-hour urinary protein excretion rate (−56% vs. baseline) more effectively than full doses of benazepril (−45.9%, $P = 0.02$) or valsartan (−41.5%, $P < 0.002$) alone (Fig. 2). Proteinuria reduction was numerically superior with benazepril than with valsartan. In 13 patients, maximal proteinuria reduction was achieved with combined treatment, in seven patients with benazepril alone, while in four patients with valsartan alone. Patients with more proteinuria reduction with combined therapy had remarkably higher basal proteinuria (4.4 ± 2.7 g/24 hours vs. 2.1 ± 2.4 g/24 hours and 2.0 ± 1.1 g/24 hours, $P < 0.01$ and $P < 0.05$, respectively). In these patients differences between the levels of proteinuria achieved with combined therapy (1.6 ± 1.8 g/24 hours) and those achieved by the other two treatments (benazepril, 2.4 ± 2.2 g/24 hours; valsartan, 2.8 ± 2.7 g/24 hours) averaged about 1 g/24 hours and were both highly significant ($P = 0.001$ vs. benazepril and $P < 0.0001$ vs. valsartan). In the other two groups, differences between most effective treatment and the others ranged between 0.3 and 0.4 g/24 hours. Changes in urinary protein/urinary creatinine followed a similar trend with maximal and minimal reductions with combined and valsartan treatment, respectively, but without significant differences between benazepril and valsartan. Serum albumin concentration increased with all treatments, the increase being more consistent with combined or benazepril than with valsartan therapy (Table 2). Total and high-density lipoprotein cholesterol decreased to a similar extent with all treatments (Table 2).

Renal hemodynamics

The GFR similarly and marginally increased during the three treatment periods. The ERPF increased during all treatment periods, but the increase during combined or benazepril therapy numerically exceeded that observed during valsartan therapy (Fig. 3). The above changes in GFR and ERPF resulted in a greater reduction in FF during combined or benazepril therapy as compared to valsartan therapy alone (Fig. 3 and Table 3). RVR decreased during all treatment periods, but the decrease during combined and benazepril was almost double compared to the decrease observed after valsartan therapy (Fig. 3 and Table 3). As expected, changes in ERPF and RVR were significantly correlated in the study group as a whole ($r = -0.737$, $P < 0.0001$) and within each treatment group (combined: $r = -0.871$, $P < 0.0001$; benazepril: $r = -0.844$, $P < 0.0001$; and valsartan: $r = -0.689$, $P < 0.0001$).

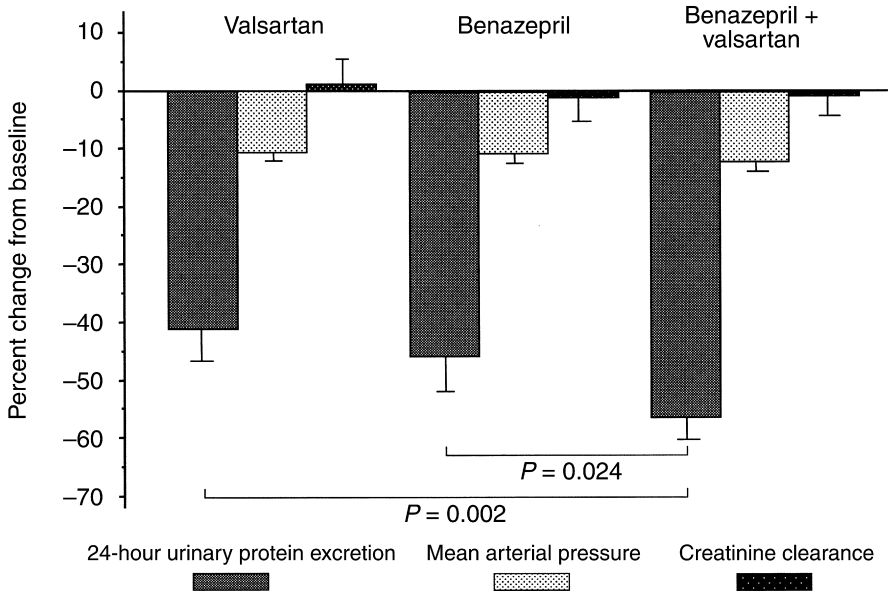

Fig. 2. Percent changes from baseline for 24-hour urinary protein excretion rate, mean arterial pressure (MAP) and creatinine clearance. With Bonferroni adjustment, the level of statistical significance is $P < 0.025$. Data are mean ± SEM.

Table 2. Clinical characteristics at randomization and at the end of each treatment period

	Randomization	Valsartan	Benazepril	Benazepril and valsartan
Systolic blood pressure *mm Hg*	140 ± 13	129 ± 12	126 ± 9	124 ± 12
Diastolic blood pressure *mm Hg*	91 ± 8	79 ± 8	80 ± 8	78 ± 9
Mean arterial pressure *mm Hg*	107 ± 8	95 ± 8	95 ± 8	94 ± 10
24 hour urinary protein *g/24 hours*	3.28 ± 2.60	2.04 ± 2.36	1.76 ± 1.88	1.39 ± 1.54[a,b]
$U_{protein}/U_{creatinine}$	1.89 ± 1.41	1.16 ± 1.25	1.03 ± 1.06	0.80 ± 0.85[a,b]
Albumin clearance *mL/min* ($\times 10^{-3}$)	489.7 ± 805.6	225.4 ± 390.4	169.5 ± 209.3	156.5 ± 248.1[b,c]
Serum creatinine *mg/dL*	1.67 ± 0.46	1.72 ± 0.42	1.72 ± 0.43	1.75 ± 0.46
Creatinine clearance *mL/min*	69.14 ± 19.86	67.88 ± 17.21	66.22 ± 15.33	67.65 ± 18.49
Serum potassium *mEq/L*	4.1 ± 0.56	4.33 ± 0.37	4.45 ± 0.39	4.63 ± 0.42[b,c]
Serum albumin *g/dL*	3.51 ± 0.63	3.69 ± 0.56	3.7 ± 0.46	3.71 ± 0.45
Hemoglobin *g/dL*	14.24 ± 1.84	13.45 ± 1.74	13.48 ± 1.76	13.1 ± 1.87[b,c]
Total cholersterol *mg/dL*	224.21 ± 35.09	208.96 ± 24.16	207.08 ± 28.02	205.88 ± 29.02
High-density lipoprotein *mg/dL*	48.21 ± 12.99	40.88 ± 13.89	41.63 ± 12.51	39.92 ± 12.35
Triglycerides *mg/dL*	177 ± 160	202 ± 181	181 ± 174	163 ± 114
24-hour urinary sodium *mEq/24 hours*	195.49 ± 59.21	191.80 ± 59.06	200.12 ± 57.13	203.55 ± 58.76

Data presented as mean ± standard deviation, paired *t* test.
[a] $P < 0.01$ compared to valsartan
[b] $P < 0.05$ compared to benazepril
[c] $P < 0.05$ compared to valsartan

Dextran clearances and theoretical analysis of glomerular dextran transport

The fractional clearances of neutral dextran molecules of radii ranging from 24 to 70 Å are shown in Figure 4. The sieving profiles at the end of combined and of benazepril or valsartan therapy were comparable.

The results of the theoretical analysis of glomerular hemodynamics and glomerular membrane permeability are listed in Table 3. This analysis requires an assumption of the transmembrane hydraulic pressure difference (ΔP), as this parameter cannot be measured in humans. Indirect evidence from healthy subjects suggests that ΔP should be close to 40 mm Hg [15]. As our study patients were hypertensive and without RAS blockade at the beginning of the study, we assumed a slightly elevated ΔP of 45 mm Hg at baseline. Because experimental evidence is available that ACEi and ARA selectively and similarly reduce glomerular capillary pressure in animals and since both normalized blood pressure to a similar extent, we assumed that both benazepril and valsartan decreased ΔP to about 40 mm Hg [19, 20]. Since the effect of combined therapy on arterial blood pressure was similar to that of the two drugs alone, and in the absence of any data in experimental animals, we assumed a ΔP of 40 mm Hg also during combined benazepril and valsartan therapy. As a result, the ultrafiltration coefficient K_f listed in Table 3 significantly and similarly increased, and the mean pore size u of the corresponding numerical distribution

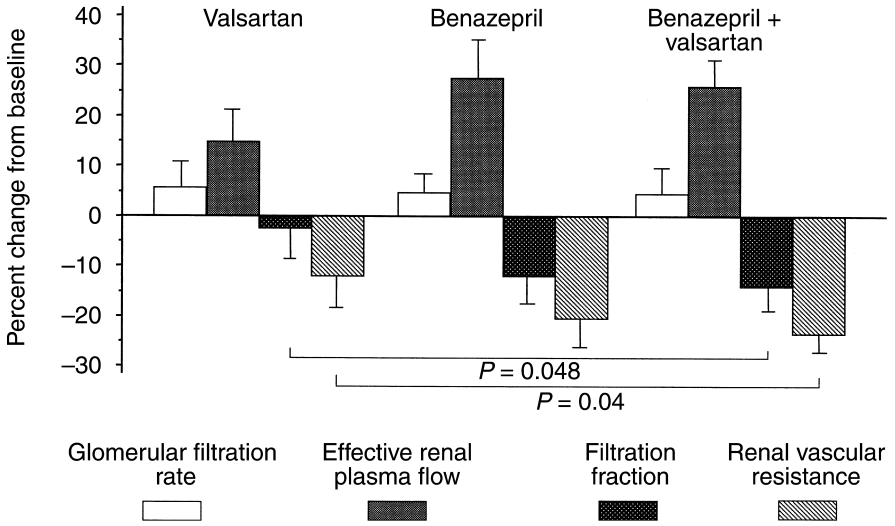

Fig. 3. Percent changes from baseline for glomerular filtration rate (GFR), effective renal plasma flow (ERPF), filtration fraction (FF), and renal vascular resistances (RVR). With Bonferroni adjustment, the level of statistical significance is $P < 0.025$.

Table 3. Renal hemodynamic parameters and glomerular membrane permeability characteristics at randomization and at the end of each treatment period

	Randomization	Valsartan	Benazepril	Benazepril and valsartan
Glomerular filtration rate *mL/min/1.73 m²*	46.5 ± 12.8	47.9 ± 14.6	47.7 ± 13.5	48.1 ± 17.1
Effective renal plasma flow *mL/min/1.73 m²*	285 ± 102	317 ± 127	349 ± 125	353 ± 129
Filtration fraction %	17.3 ± 4.9	16.3 ± 5.1	14.7 ± 4.6	14.3 ± 4.2
Kf *mL/min/1.73 m²*	2.61 ± 0.91	4.38 ± 3.41	4.27 ± 2.48	4.14 ± 2.34
u *Å*	52.62 ± 8.51	48.33 ± 7.13	51.53 ± 9.16	50.42 ± 11.12
s *Å*	1.19 ± 0.09	1.22 ± 0.07	1.19 ± 0.08	1.19 ± 0.1
$\omega_o \times 10^{-3}$	3.22 ± 2.9	1.35 ± 1.96	1.24 ± 1.61	2.02 ± 3.30
Sum of squared errors	0.97 ± 0.89	0.93 ± 0.78	1.12 ± 1.28	1.41 ± 1.39

Data presented as mean ± standard deviation, paired *t* test

similarly decreased during each of the three treatment periods as compared to baseline. No change occurred in the standard deviation throughout each treatment period. The shunt parameter, ω_o, a quantitative measure of the importance of the nonselective pore population, which does not restrict the passage of largest macromolecules, including circulating plasma proteins, similarly decreased during each treatment period versus baseline (Table 3).

Repeated measures ANOVA mixed models

At univariate analysis there was a borderline significant relationship between RVR and log-transformed 24-hour urinary protein excretion rate ($P = 0.087$). No relationship was found between GFR ($P = 0.947$) or ω_o ($P = 0.694$) and 24-hour urinary protein excretion. However, when the analysis accounted for the borderline interaction between RVR and GFR ($P = 0.089$), GFR-adjusted RVR were significantly associated with 24-hour urinary proteins ($P = 0.046$).

Safety

The study treatments were well-tolerated by all patients. No major change in serum creatinine and creatinine clearance occurred throughout the whole study period (Table 2). Serum potassium similarly increased during each treatment period but the increase did not exceed 0.5 mEq/L versus baseline and no patient required reduction of drug dose or cessation of therapy because of hyperkalemia. Changes in hemoglobin were not associated with symptoms of anemia and did not require therapy.

DISCUSSION

The results from this study demonstrate that 8 weeks of combined therapy with half doses of benazepril and valsartan reduced proteinuria more effectively than 8 weeks treatment with full doses of benazepril or valsartan. The benefit of combined therapy was more consistent, and clinically relevant, in patients with more severe, nephrotic-range, proteinuria. In patients with subnephrotic proteinuria, differences between treatments were negligible and probably reflected random fluctuations more than a real superiority of one treatment versus the others. The superior antiproteinuric effect was not dependent on greater blood pressure reduction with combined or benazepril therapy, as blood pressure control was virtually identical during each treatment period. Nor did the

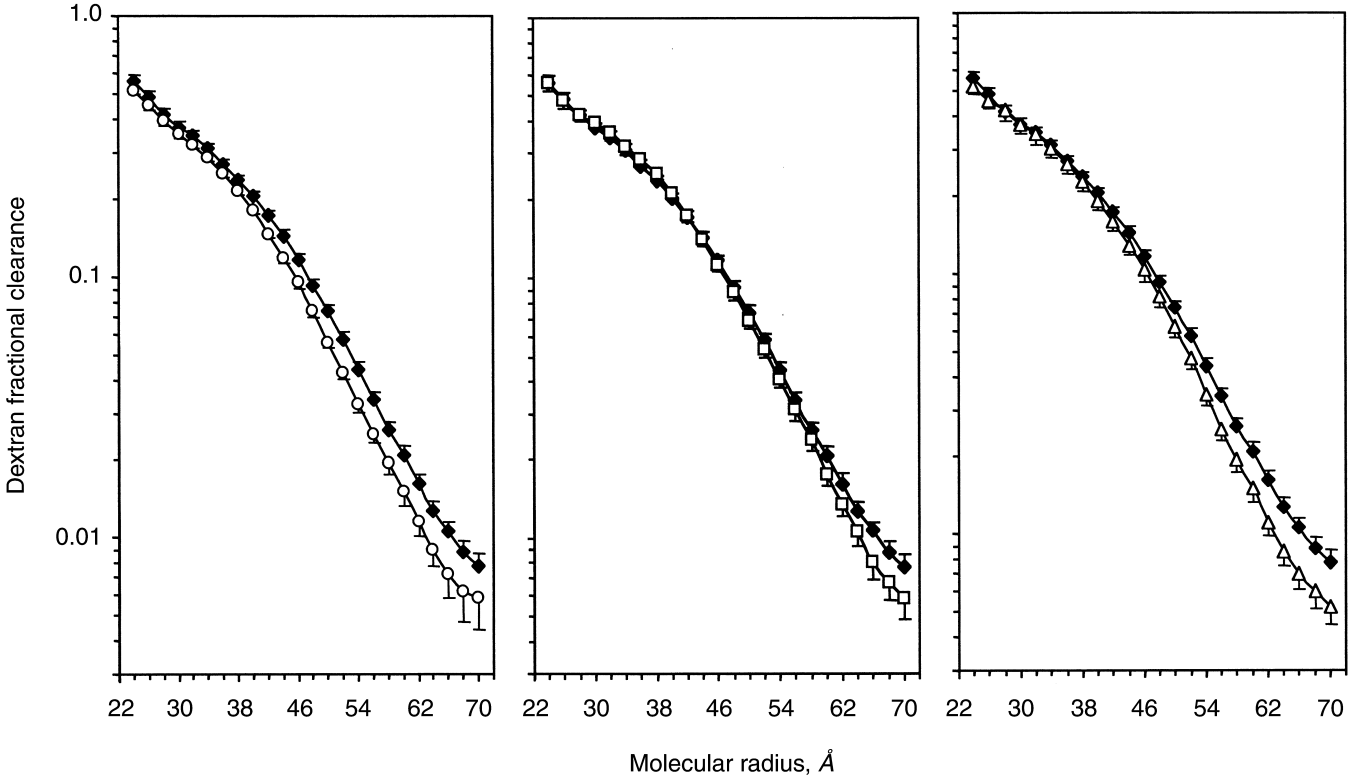

Fig. 4. Fractional clearances of neutral dextrans at baseline and at the end of the treatment periods with valsartan, benazepril, and benazepril and valsartan.

effect depend on further amelioration of the glomerular barrier size selectivity properties, since combined therapy and the therapy with either of the two drugs alone had virtually identical effects on the shunt pathway and on pore radii distribution.

A particular strength of the present study was that for the first time the potential additional/synergistic effect of combined therapy on urinary proteins was assessed without the confounding effect of arterial blood pressure. Previous studies in diabetic patients with micro- [9] or macro- [21] albuminuria and nondiabetic patients with proteinuria [8] found that more proteinuria reduction achieved with combined therapy was almost invariably associated with more blood pressure reduction. Thus, these studies did not address the central issue of a potential additional or synergistic antiproteinuric effect of combined therapy possibly sustained by a degree of RAS inhibition that cannot be achieved by single ACEi or ARA treatments, even at full recommended doses. Of note, the doses of benazepril and valsartan used as single therapy in the present study were equivalent to doses of ACEi and ARA previously found to achieve maximal (and comparable) reduction in proteinuria [7, 22]. On the other hand, a previous study found that when low doses of losartan (50 mg/day) were added on high doses of lisinopril (40 mg/day), urinary proteins were not further reduced by combined therapy versus ACE inhibition alone [23]. Thus, whether combined therapy may also increase the antiproteinuric effect of maximized RAS inhibition achieved by high doses of ACEi or ATA alone is still matter of investigation.

Measuring size selective function by the fractional clearance of graded-size neutral dextran molecules was instrumental to quantify and compare the effective amelioration of glomerular barrier permeability achieved by benazepril, valsartan, or their combination and to assess to which extent this could account for the different effect on urinary proteins observed with the three regimens. The best fitting of fractional clearance data was obtained considering the "log-normal + shunt" model of glomerular size selectivity. The model showed that the different treatments, in addition to similarly decreasing the mean radius of pores that cross the glomerular barrier, also had a virtually identical effect on the shunt parameter ω_o, (Table 3). This finding was consistent with a similar direct effect on glomerular membrane size selective function and did not explain the more effective reduction in urinary proteins achieved by combined benazepril and valsartan therapy, as compared to benazepril or valsartan single therapy.

We did find that, compared to baseline, the ERPF increased with all treatments, the increase being maximal

and comparable with combined and benazepril treatment and quite less consistent (about 50% lower) with valsartan single treatment. Due to the marginal and comparable changes in GFR, this resulted in changes in FF that followed a similar trend (Fig. 3). Finding that the increase in ERPF observed in combined and benazepril group strongly correlated with RVR reduction strongly suggest that the increased kidney perfusion depended on an intrarenal vasodilation largely sustained by the inhibition of the converting enzyme. In the study group as a whole, reduction in RVR, but not in ω_o, predicted the reduction in proteinuria. This suggests that the better antiproteinuric effect of combined therapy and, albeit to a less extent, benazepril alone, as compared to valsartan therapy could, at least in part, depend on a hemodynamic phenomenon that did not affect glomerular barrier size selectivity and plasma protein ultrafiltration. More proteinuria reduction despite comparable glomerular sieving function may depend on increased tubular protein reabsorption. A working hypothesis is that in the present experimental setting, increased protein reabsorption reflected an improved tubular function sustained by an improved perfusion, as documented by increased renal blood flow and decreased RVR during combined or ACEi therapy. This hypothesis is consistent with experimental evidence that several of the effects of ACEi that may contribute to renal protection have been attributed to the associated rise in kinins resulting in predominant efferent arteriole dilation and consequent increase in renal blood flow [24–27].

Regardless of the involved mechanisms, the finding that combined therapy reduced urinary protein excretion rate more than either benazepril and, more consistently, than valsartan alone has major clinical implications. Reduction in proteinuria is an important goal in the treatment of chronic nephropathy. The importance of decreasing proteinuria was demonstrated by multiple experimental studies showing that early and persistent proteinuria reduction invariably limits or prevents glomerular and tubulointerstitial damage [28]. Likewise, many prospective, randomized trials in diabetic [29, 30] and nondiabetic [31] chronic nephropathies invariably found that a decrease in proteinuria in response to ACEi or ARA therapy predicted a slowing of the loss of GFR over time and of the progression to ESRD [2]. In animal studies, proteinuria has been linked to an increase in inflammatory cytokines and mediators such as nuclear factor-κB (NF-κB), endothelin-1, monocyte chemoattractant protein-1 (MCP-1) and regulated upon activation, normal T cell expressed and secreted (RANTES) [28]. Activation of such mediators can lead to interstitial fibrosis and scarring, and limiting or preventing this sequence of events by reducing protein traffic may eventually preserve renal structure and function [32, 33].

Overall, the combination of ACEi and ARA was well-tolerated. There were no significant side effects associated with the therapy. No patient developed hyperkalemia that mandated withdrawal from the protocol or treatment with potassium-binding resins. Serum hemoglobin levels were significantly lower in the combined treatment period, which was clinically insignificant, and did not require therapy. There was a significant and comparable decrease in high-density lipoprotein cholesterol during each treatment period that, however, was accompanied by a decrease also in total cholesterol. In the present study, the reduction in proteinuria was not associated with a concomitant decrease in GFR. Of note, patients were on an unrestricted water and sodium intake, and temporarily withdrew diuretic therapy before the first administration of ACEi or ATA. This prevented the potentiating effect of hypovolemia on acute, hemodynamically mediated GFR decline observed in patients given low sodium diet or diuretic therapy to enhance the antiproteinuric effects of RAS inhibition [34].

CONCLUSION

In conclusion, we found that in chronic nondiabetic nephropathies the combination of low dose benazepril and valsartan was safe and well-tolerated. Combined therapy better reduced proteinuria than either agent alone. There was also a trend to greater proteinuria reduction with benazepril than with losartan alone, but the study was not powered to detect this difference. The different effect on urinary proteins was not dependent on different effects on blood pressure or glomerular barrier size selectivity, but was most likely related to a hemodynamic effect specifically related to the inhibition of the ACE. These findings call for randomized trials intended to assess whether the antiproteinuric and hemodynamic effects of combined ACEi and ARA may in the long-term confer more renoprotection than the two treatments alone, even at comparable blood pressure control. These trials should probably include patients with more severe, nephrotic, proteinuria who still have a poor prognosis on ACEi or ARA alone, but who also seem to benefit the most from the superior antiproteinuric effect of combined therapy.

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