ACE inhibition and ANG II receptor blockade improve glomerular size-selectivity in IgA nephropathy

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1Department of Kidney Research, Mario Negri Institute for Pharmacological Research, 24125 Bergamo; 2Unit of Nephrology and Dialysis, Ospedali Riuniti di Bergamo, Bergamo; and 3Unit of Nephrology and Dialysis, Ospedale Treviglio-Caravaggio, 24047 Treviglio, Italy

Remuzzi, Andrea, Norberto Perico, Fabio Sangalli, Giovanni Vendramin, Monica Moriggi, Piero Ruggenenti, and Giuseppe Remuzzi. ACE inhibition and ANG II receptor blockade improve glomerular size-selectivity in IgA nephropathy. Am. J. Physiol. 276 (Renal Physiol. 45): F457–F466, 1999.—Protein trafficking across the glomerular capillary has a pathogenetic role in subsequent renal damage. Despite evidence that angiotensin-converting enzyme (ACE) inhibitors improve glomerular size-selectivity, whether this effect is solely due to ANG II blocking or if other mediators also play a contributory role is not clear yet. We studied 20 proteinuric patients with IgA nephropathy, who received either enalapril (20 mg/day) or the ANG II receptor blocker irbesartan (100 mg/day) for 28 days in a randomized double-blind study. Measurements of blood pressure, renal hemodynamics, and fractional clearance of neutral dextran of graded sizes were performed before and after 28 days of treatment. Both enalapril and irbesartan significantly reduced blood pressure over baseline. This reduction reached the maximum effect 4–6 h after drug administration but did not last for the entire 24-h period. Despite transient antihypertensive effect, proteinuria was effectively reduced by both treatments to comparable extents. Neither enalapril nor irbesartan modified the sieving coefficients of small dextran molecules, but both effectively reduced transglomerular passage of large test macromolecules. Theoretical analysis of sieving coefficients showed that neither drug affected significantly the mean pore radius or the spread of the pore-size distribution, but both importantly and comparably reduced the importance of a nonselective shunt pathway. These data suggest that angiotensin II is the key mechanism by which ACE inhibitors exert their beneficial effect on glomerular size-selective function and consequently on glomerular filtration and urinary output of plasma proteins.

angiotensin II; angiotensin II receptor antagonism; dextran fractional clearance; glomerular size-selectivity; immunoglobulin A; proteinuria

ANIMALS AND HUMANS with proteinuric nephropathies, either of diabetic or nondiabetic origin, tend to develop renal structural damage associated with progressive renal function decline over time. Studies of animals have disclosed that glomerular adaptations to the loss of functioning renal mass, such as hypertrophy and concomitant increase in glomerular capillary pressure and flow, are common denominators of progressive nephropathies after an initial insult has reduced the number of functioning nephrons (2, 12). Evidence is also available from studies of experimental animals that protein trafficking across the glomerular capillary has a pathogenetic role in subsequent renal damage via the nephritogenic insult generated by the process of tubular endocytosis of filtered proteins (5–7, 15, 42). Moreover, an analysis of the most recent studies allows one to conclude that proteinuria was a major determinant of the rate at which renal function is lost in human renal diseases (10, 35, 44, 46). This body of evidence suggests that glomerular hemodynamic and membrane permeability changes are related to each other to the extent that enhancing intraglomerular capillary pressure enlarges pore radii in various models, including renal vein constriction (52) and immune nephropathies (53).

That ANG II mediates glomerular permselective function via the opening of large unselective pores after elevations in transmembrane pressure differences is consistent with findings of enhanced fractional clearance of large dextran macromolecules in the isolated perfused kidney (27) and in animal models in vivo (9). Angiotensin-converting enzyme (ACE) inhibitors, via their unique property of reducing membrane pore dimensions (40) thereby improving sieving function, are more renoprotective than other drugs in animal models of renal disease progression, from diabetic to immune or toxic models (1, 3, 55), and limit decline of glomerular filtration rate (GFR) in human diabetic (28) and nondiabetic (19, 29) renal diseases. The protective effect of ACE inhibitors appears, however, confined to patients with high values of urinary proteins (i.e., >2–3 g/24 h) in most trials (19, 29). Furthermore, some studies have found that ACE inhibitors, and possibly other antihypertensives, limit subsequent renal function decline to the extent that they lower proteinuria (8, 25). Despite this experimental and human evidence that ACE inhibitors improve glomerular size-selectivity via their properties of interfering with the renin-angiotensin axis, whether the protective effects of the above class of compounds is solely due to ANG II blocking or if other mediators or hormonal systems may also play a contributory role is still far from clear. ACE inhibitors do block a number of other systems, including the bradykinin system, that are implicated in renal damage (22–24, 49). Specifically, an ACE inhibitor, but not an ANG II receptor antagonist, reduced proteinuria in early aminonucleoside nephrosis, an effect that was not observed when a bradykinin antagonist was simultaneously administered (50). Moreover, infusion of ANG II failed to reverse lisonopril’s effect of improving
size-selective function in patients with nondiabetic chronic renal disease (21). The availability of receptor antagonists that effectively and selectively prevent ANG II binding to AT1-type receptors without altering other hormonal systems provides an opportunity to test in humans whether inhibition of the local action of ANG II reversed size-selective dysfunction and acutely reduced proteinuria, a valuable predictor of long-term protection toward declining GFR (44, 45). We recently had the opportunity to study a group of patients with IgA nephropathy, who were randomized to receive enalapril or irbesartan for 28 days in a double-blind study of two parallel groups (33). This was a sequential multidrug study mainly designed to address whether an additional drug (in this case indomethacin) added after either treatment could further reduce proteinuria. In this group of patients, sequential measures of blood pressure and evaluation of renal hemodynamics and intrinsic glomerular membrane permeability properties to macromolecules before and after either drug allowed us to compare the antihypertensive effect with glomerular hemodynamics and intrinsic glomerular membrane permeability properties to macromolecules. The results of these studies form the basis of this report.

METHODS

Patient population. Twenty patients, 16 males and 4 females aged 20–65 yr with biopsy-proven IgA mesangial nephropathy, were enrolled into the study. The patients were recruited from the outpatient clinic of the Unit of Nephrology of Ospedali Riuniti (Bergamo, Italy) and Ospedale Treviso-Caravaggio (Treviso, Italy). Patients were persistently proteinuric (0.5–4.0 g urinary proteins/24 h) with normal or moderately reduced renal function (serum creatinine 0.9–2.4 mg/dl). No previous or concomitant immunosuppressive treatments and no nonsteroidal anti-inflammatory drugs were used in the 3 mo before enrollment. Informed consent from each patient was obtained before entry into the study. Twelve patients were normotensive, and the remaining patients were on conventional antihypertensive treatments. Antihypertensive treatment was stopped before the selection visit was performed. Patients were randomized to receive the ACE inhibitor enalapril (20 mg/day; Sanofi Winthrop, Gentilly Cedex, France) or the ANG II receptor antagonist irbesartan (100 mg/day; Sanofi Winthrop) in a double-blind study with two parallel groups. The irbesartan dose was chosen according to a previous dose-finding study of lowering blood pressure in patients with essential hypertension. Study protocol. After enrollment in the study, a 4-wk single-blind placebo washout period from previous treatments was performed (33). One week before the end of this washout period, patients underwent 24-h protein excretion measurements and a renal clearance study (baseline) to evaluate GFR, renal plasma flow (RPF), and fractional clearance of albumin. At the end of the placebo phase, patients were randomized to enalapril or irbesartan and continued the assigned treatment for the following 28 days, after which urinary protein excretion was measured and a second clearance study was performed with the same modality of baseline evaluation.

Clearance studies. Inulin and para-aminohippuric acid (PAH) clearance were measured under a steady state of water diuresis induced by oral water loading as previously described (33, 39). Briefly, primed infusion of inulin and PAH was followed by slow intravenous administration of neutral dextran test macromolecules (130 mg/kg, Dextran-40, Rheomacrodex; Pharmacia, Uppsala, Sweden) after ~15 min. A sustained infusion of inulin and PAH was then started to maintain constant plasma concentrations of both tracers. Drug or placebo was administered 30 min after the start of the priming infusion of inulin and PAH. After an equilibration period of ~60 min, a timed urine collection of ~30 min was made by spontaneous voiding for evaluation of dextran fractional clearance. Subsequently, six clearance periods of 2 h each were performed to monitor eventual changes in renal hemodynamics and albumin fractional clearance acutely induced by single drug administration. Blood samples were collected at the beginning and end of each clearance period. Blood pressure was measured every 2 h during the entire clearance study. Urine and plasma samples obtained during the first clearance period were used to determine fractional clearance of dextran molecules. In the same urine and plasma samples, albumin concentration was also measured.

Inulin and PAH concentrations in plasma and urine samples were determined with colorimetric assays as previously described (33, 39). Separation of graded-size dextran molecules was achieved by gel permeation chromatography on a Sephacryl S-300 column (1.6 × 95 cm) using dextran standards of known molecular weight (Pharmacosmes, Viby Sj., Denmark) for column calibration. Molecular radii of individual dextran fractions were calculated according to Oliver et al. (32). Dextran concentrations in eluted fractions were determined using the anthrone reaction as previously described (39, 41, 48). Fractional clearance of dextran molecules was computed as

$$\Theta_D = \frac{(U/P)_D / (U/P)_I}{(U/P)_D / (U/P)_I}$$

where (U/P)_D and (U/P)_I are the urine-to-plasma concentration ratios of dextran and inulin, respectively. Total protein concentration in plasma samples collected during the first clearance period and in urine samples collected over the 24-h period were measured by an automatic analyzer (Synchrom CX5; Beckman, Furlenton, CA). Albumin concentration in plasma and urine samples collected during the clearance studies was determined by nephelometric technique (Beckman; detection limit of assay is 3 µg/ml of albumin in urine samples). GFR and RPF were calculated as inulin and PAH clearance, respectively, and normalized for body surface area. To take into account that extraction of PAH during renal passage is not complete in these patients (4), we assumed a renal extraction coefficient of 0.8 and 0.7, respectively, for patients with GFR higher or lower than 80 ml·min⁻¹·1.73 m⁻².

Theoretical analysis of glomerular membrane transport. We investigated intrinsic glomerular membrane permeability properties of macromolecules using the mathematical model of glomerular size-selectivity described in detail previously (13, 36, 41). 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 lognormal probability distribution. In parallel with selective pores, a shunt pathway consisting of large pores that do not restrict the passage of large test macromolecules is also assumed (13, 41). This distribution of pore radii is therefore characterized by three adjustable parameters: u, s, and w₀. The parameters u and s represent, respectively, the mean and the standard deviation of the corresponding normal probability distribution, and w₀...
represents the fraction of ultrafiltrate that would pass through the shunt if plasma protein were absent (13, 41). 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 (14). The intrinsic membrane permeability parameters were calculated as shown previously (39, 41), and the sum of squared errors between experimental and calculated sieving coefficients was minimized at single patient level during each clearance study.

Statistical analysis. Data are expressed as means ± SD or median and range, as specified. Results were analyzed using two-way ANOVA, and specific comparisons among different groups were performed by two-tailed Student's t-test using the Bonferroni correction (51). Values of urinary protein excretion and albumin fractional clearance were log-transformed before statistical analysis. Statistical analysis was performed using the software package StatView (Abacus Concepts, Berkeley, CA).

RESULTS

Blood pressure and kidney function. The effects of 4-wk treatment with enalapril or irbesartan on systemic and renal hemodynamic parameters are summarized in Table 1. Values of renal hemodynamic parameters and albumin fractional clearance are the average of the six clearance periods. The randomization process generated two groups: 11 patients in the enalapril group, 9 in the irbesartan group. The two groups were statistically different for blood pressure, urinary protein excretion, and albumin fractional clearance at baseline. In patients treated with enalapril, albumin pressure and urinary proteins were lower than in irbesartan-treated patients. For this reason, comparisons of the effects of the two drugs were performed considering the relative changes induced by individual treatments as well as by direct comparison of absolute values of end-point parameters before and after treatment (see Table 1).

As shown in Table 1, blood pressure did not change significantly during the time course of baseline (placebo) evaluation but was effectively reduced during the clearance study performed at the end of the treatment period. The maximum antihypertensive effect was observed 4–6 h after drug administration. At this time, mean blood pressure was slightly but significantly lower at the end of enalapril or irbesartan treatment than at baseline evaluation. By contrast, trough levels of blood pressure (measured before drug administration) were only numerically reduced for both treatments; mean blood pressure (in mmHg) averaged 101 ± 9 vs. 96 ± 9 for baseline vs. enalapril, and 112 ± 8 vs. 110 ± 7 mmHg for baseline vs. irbesartan. These differences did not reach statistical significance.

The mean GFR values of the six clearance periods were not affected significantly by 28 days of enalapril treatment (see Table 1). Similarly, mean values of RPF at the end of the treatment period were only numerically higher than at baseline; the differences did not reach statistical significance. In patients given irbesartan, the mean values of GFR were comparable at baseline evaluation and after treatment. By contrast, mean RPF was significantly elevated by drug treatment. As a result, mean filtration fraction remained constant in enalapril-treated patients, whereas it decreased significantly at day 28 of treatment in those given irbesartan. Urinary excretion rate of total proteins and albumin as well as albumin fractional clearance decreased significantly with enalapril treatment (see Table 1). Irbesartan treatment significantly reduced urinary excretion of total proteins and albumin but, unlike enalapril, only numerically lowered albumin fractional clearance.

The dynamics of mean blood pressure changes induced by enalapril and irbesartan during the second clearance study is reported in Fig. 1. In enalapril-treated patients, mean blood pressure was significantly reduced during the clearance study 4–10 h after drug administration compared with values measured before drug administration; then blood pressure returned to preadministration level. These changes in blood pressure were not associated with glomerular hemodynamic changes such as RPF, nor with acute changes in albumin fractional clearance during the course of the 12-h observation period. Similarly, in irbesartan-treated patients, mean blood pressure transiently and

| Table 1. Blood pressure and kidney functional parameters |
|-----------------|-----------|-----------|-----------------|-----------|-----------|
|                  | Enalapril  |           |                  | Ibesartan  |           |
|                  | Baseline   | End of treatment | Mean % change | Baseline   | End of treatment | Mean % change |
| SBP, mmHg        | 133 ± 9    | 124 ± 10*   | 6.4%           | 147 ± 13   | 142 ± 12   | 3.0%           |
| DBP, mmHg        | 79 ± 12    | 69 ± 9†     | 12.4%          | 91 ± 11    | 82 ± 8†    | 9.1%           |
| MBP, mmHg        | 97 ± 10    | 87 ± 8†     | 9.7%           | 109 ± 11   | 102 ± 8†   | 6.4%           |
| GFR, ml·min⁻¹·1.73 m⁻² | 66 ± 19    | 65 ± 25     |                | 54 ± 15    | 55 ± 11    |                |
| RPF, ml·min⁻¹·1.73 m⁻² | 537 ± 156  | 585 ± 177  |                | 430 ± 110  | 549 ± 117† |                |
| FF, %            | 12 ± 3     | 11 ± 3      |                | 13 ± 3     | 10 ± 1*    |                |
| Ur Prot, g/24 h  | 1.44 ± 1.11| 0.72 ± 0.39*| 38.6%          | 2.48 ± 2.02| 1.54 ± 1.46| 45.4%          |
| Ur alb, g/24 h   | 1.27 ± 0.90| 0.55 ± 0.30†| 49.0%          | 1.92 ± 1.42| 1.11 ± 1.09†| 49.3%          |
| θab > 10 (range) | 12.6 (4–324)| 9.31 (2–98) | 50.4%          | 34.6 (3–217)| 24.5 (6–96) | 45.5%          |
| Na⁺ excretion, mEq/24 h | 155 ± 54  | 174 ± 67    |                | 187 ± 61  | 177 ± 44   |                |

Values are represented as means ± SD or as median (range). Measurements of blood pressure values were performed 4–6 h after drug administration. SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; GFR, glomerular filtration rate; RPF, renal plasma flow; FF, filtration fraction; Ur Prot, urinary protein excretion; Ur alb, urinary albumin excretion; θab, fractional clearance of albumin. *P < 0.05 vs. same group at baseline. †P < 0.01 vs. same group at baseline.
significant decreased with time at 4 and 6 h after drug administration compared with values measured before administration; then blood pressure returned to preadministration values. Also in this patient group, acute change in blood pressure was not associated with significant changes in RPF and albumin fractional clearance. Thus the adopted doses of the two treatments acutely and transiently reduced mean blood pressure but did not induce acute changes in glomerular plasma flow rate and urinary albumin excretion.

Neutral dextran fractional clearance. Sieving coefficients of neutral dextran molecules of graded sizes (26–66 Å) measured in basal condition and at the end of enalapril or irbesartan treatment are reported in Table 2 and Fig. 2. Fractional clearance of small dextran molecules (radii < 42 Å) was not significantly affected by enalapril treatment. Sieving coefficients of larger dextran molecules (radii 42–44 and 64–66 Å) were significantly lower after enalapril treatment than at baseline. Similarly, irbesartan administration had no effect on fractional clearance of small dextrans (radii < 38 Å) but significantly reduced the sieving coefficient of molecules of larger size (38–50 and 54–66 Å).

Dextran sieving coefficients and renal hemodynamic parameters measured during the first clearance period were used as input data for the theoretical analysis of glomerular size-selective function. This theoretical approach requires the assumption of glomerular transmembrane hydraulic pressure difference (ΔP) that cannot be directly measured in humans. In keeping with previous studies (31, 39), we assumed that ΔP = 45 mmHg in these patients at baseline conditions, a value slightly elevated above what is believed to be a normal value (40 mmHg) to take into consideration the moderate hypertension that characterizes our patient population. Because in experimental models of glomerular disease ACE inhibitors and ANG II receptor blockers have been shown to selectively decrease glomerular capillary pressure (26, 30), we assumed a value of ΔP = 40 mmHg for theoretical analysis of sieving coefficients measured at the end of the treatment period. In addition, because of the uncertainty in estimating representative values of ΔP, we also considered the possibility that despite antihypertensive treatments, glomerular capillary pressure was unchanged, and we performed
related to the assumption of a lower
ment compared with baseline, but this effect was
significantly higher after enalapril and irbesartan treat-
membrane pore-size parameters. The same statisti-
mmHg) were less important for the other calculated
ation. The effects of the assumed
mmHg). With the assumption of
values of \( \Delta P \) do not
importantly affect calculated membrane pore-size pa-
tained constant (see Table 3). This is in keeping with
previous observations that assumed values of \( \Delta P \) were
structurally significant changes in these parameters between
baseline and end-of-treatment evaluation were com-
puted assuming either that \( \Delta P \) was reduced or that it
remained constant (see Table 3). This is in keeping with
previous observations that assumed values of \( \Delta P \) did not
attain statistical significance. In Fig. 3 we
graphically represented the effect of the two treatments
on the pore-size distribution function \( g(r) \) previously
described (41). Values of \( g(r) \) as a function of pore
radius were calculated using mean values of \( u \) and \( s 
reported in Table 3 (assuming \( \Delta P = 40 \) mmHg at
the end of both treatment periods). Enalapril and irbesar-
tan similarly reduced the radius of the restrictive
membrane pore population. We also calculated the
membrane pore parameter \( \omega_0 \), which describes the
relative importance of the shunt pathway and repres-
ts the filtration volume fraction that would pass
through the shunt if plasma proteins were absent. An
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age, \( \omega_0 \) was reduced by more than 55 and 40% by
enalapril treatment for \( \Delta P = 40 \) and 45 mmHg, respec-
tively, compared with basal evaluation (Table 3). Simi-
larly, irbesartan reduced the \( \omega_0 \) by more than 49 and
43% for \( \Delta P = 40 \) and 45 mmHg, respectively, compared
with baseline. As shown in Fig. 4, individual changes in
shunt parameter at the end of enalapril or irbesartan
treatment paralleled those of 24-h urinary protein
excretion rate.

### Table 2. Fractional clearance of neutral dextran molecules of graded sizes

<table>
<thead>
<tr>
<th>Radius ( Å)</th>
<th>Enalapril Baseline</th>
<th>End of treatment</th>
<th>Irbesartan Baseline</th>
<th>End of treatment</th>
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<tr>
<td>26</td>
<td>0.84±0.16</td>
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Values are means ± SD.

Theoretical analysis of sieving coefficients at the end of
the treatment period assuming \( \Delta P = 45 \) mmHg.

The results of the theoretical analysis are reported in
Table 3 and in Fig. 3. Calculated values of \( K_v \) were
significantly higher after enalapril and irbesartan treat-
ment compared with baseline, but this effect was
related to the assumption of a lower \( \Delta P \) value (40
mmHg). With the assumption of \( \Delta P = 45 \) mmHg, \( K_v \) was
comparable at baseline and at end-of-treatment evalua-
tion. The effects of the assumed \( \Delta P \) values (40 vs. 45
mmHg) were less important for the other calculated
membrane pore-size parameters. The same statisti-

graphically represented the effect of the two treatments
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with baseline. As shown in Fig. 4, individual changes in
shunt parameter at the end of enalapril or irbesartan
treatment paralleled those of 24-h urinary protein
excretion rate.

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**Fig. 2.** Fractional clearance of neutral dextran macromolecules as a function of effective molecular radius measured at baseline and end of treatment period. *P < 0.05 vs. end of treatment in same group. **P < 0.01 vs. end of treatment in same group.
Data collected during the study also allowed us to compare the antihypertensive effect observed during the treatment period with the corresponding reductions in urinary proteins and glomerular size-selective parameters at the level of individual patients, independently from the treatment used to obtain different degrees of blood pressure reduction. As represented in Fig. 5, percent changes in urinary protein excretion induced by the 28-day treatment with either enalapril or irbesartan did not correlate with corresponding changes in mean blood pressure over the same treatment period. This same situation was observed comparing calculated changes in the shunt parameter $\omega_0$ (for $\Delta P = 40 \text{ mmHg}$) and corresponding changes in mean blood pressure during the treatment period.

**DISCUSSION**

The results of the present study show that in patients with IgA nephropathy and normal or moderately impaired GFR, 4-wk treatment with conventional doses of either enalapril (20 mg/day) or irbesartan (100 mg/day) significantly reduced mean blood pressure over baseline. This reduction in blood pressure was, however,
transient, reaching the maximum effect 4–6 h after
drug administration; it did not last for the entire 24-h
period. Trough levels of blood pressure did not change
significantly during both treatments. Also, renal hemo-
dynamic parameters did not change significantly after
28 days of treatment, and no statistically significant
changes in GFR were observed between values mea-
sured before and after treatment. A mild elevation in
RPF was selectively induced by irbesartan but not by
enalapril treatment. This is in keeping with previously
reported data on the effect of ACE inhibition and ANG
II receptor antagonism on renal hemodynamics (11,
17, 18).

Despite the transient antihypertensive effect during
the 24-h period, urinary protein excretion was effec-
tively reduced by both treatments, to extents compa-
rable to each other. Reductions in urinary protein
excretion over baseline values averaged 38.6 and 45.4%,
respectively, for enalapril and irbesartan. Measure-
ment of size-selective function by the fractional clear-
ance of graded-size neutral dextran molecules is instru-
mental to quantifying the effective amelioration of
glomerular membrane barrier permeability by both
drugs, which can explain how present treatments re-
duced protein excretion. A previous study by our group
(39), in which patients with IgA nephropathy were
studied sequentially before and after treatment with
enalapril, established that ACE inhibition in this dis-
ease ameliorated urinary protein excretion via a pri-
mary action on intrinsic glomerular membrane perme-
ability to macromolecules. In this study, enalapril did
not affect the sieving coefficient of small, relatively
permanent dextran molecules (radii < 42 Å) but signifi-
cantly reduced that of larger dextran macromolecules
(radii > 56 Å). That such amelioration translated into
reduction of glomerular filtration of circulating pro-
teins and in final urinary excretion was suggested by
findings of effective reduction in mean dimensions of

Fig. 4. Changes in urinary protein excretion and in
shunt parameter values associated with drug treat-
ment at individual patient level. *P < 0.05 vs.
baseline in same group. **P < 0.01 vs. baseline in
same group.

Fig. 5. Percent changes in urinary protein
excretion and in shunt parameter as a
function of mean blood pressure (MBP)
change induced by treatment either with
enalapril or irbesartan.
the largest pores, which are responsible for protein filtration (39), induced by the drug administration. This previous study only allowed us to speculate on the possibility that, in IgA nephropathy, enalapril improved glomerular barrier size-selectivity by preventing the formation of ANG II. This issue was explicitly addressed by the current investigation, the results of which, in harmony with previous experimental findings (40), first showed that neither drug modified the sieving coefficient of small permeable neutral dextrans, but both effectively reduced transglomerular passage of larger macromolecules in this pathological condition in humans.

To quantify effective changes in intrinsic membrane permeability properties induced by both treatments, we used a theoretical model of glomerular size-selectivity. This model assumes that the glomerular membrane is perforated by a restrictive pore population having lognormal probability distribution of their radii and a nonselective shunt pathway in parallel. We preliminarily verified that simulation of fractional clearance data was more precise considering the lognormal + shunt model, compared with the assumption of a lognormal distribution alone, which we have used in other studies (36, 39). Using the lognormal distribution alone, we calculated higher values of the mean sum of squared errors between measured and calculated sieving coefficient (data not shown) than we did using the lognormal + shunt model.

The effect of both drugs was to decrease in average the mean pore radius and the spread of the lognormal distribution (see Table 3 and Fig. 3), although this tendency did not reach statistical significance. By contrast, both treatments significantly lowered the importance of the shunt pathway; in fact, the shunt parameter \( P \) decreased in average >40 and 43% after enalapril and irbesartan treatment, respectively (in the conservative hypothesis, the \( \Delta P \) was not changed by the treatments). These data would suggest once again that antagonism of ANG II is the key mechanism by which ACE inhibitors exert beneficial effects on glomerular size-selective function and consequently on glomerular filtration and urinary output of plasma proteins. In our present study, we directly measured size-selectivity but not charge-selectivity of the glomerular membrane, because technical problems make the measure of this latter function difficult in humans (20). In addition, recent experimental evidences would suggest that the size of selective membrane pores under normal conditions is smaller than the size of albumin (32), indicating that size-selective dysfunction must be present for abnormal filtration of this protein. This observation would support the conclusion that the improvement of glomerular size-selectivity we have observed with the two treatments in this study is a sign of effective amelioration of permselective function toward circulating proteins. On the other hand, the observed changes in fractional clearance of large dextran molecules cannot account entirely for the more important reductions in fractional clearance and absolute excretion rate of albumin. Because it is unlikely that major differences in proximal tubular handling of albumin are induced by the treatments, the extent of the amelioration of albumin excretion suggest that, in addition to the size-selective function, the charge-selective function of the glomerular membrane must also have been improved by both antihypertensive treatments.

That both enalapril and irbesartan exert a similar quantitative effect of ameliorating membrane sieving coefficient and reducing urinary protein excretion to a comparable extent in patients with IgA nephropathy is consistent with previous animal experiments showing that both an ACE inhibitor and a selective ANG II receptor (AT\( _1 \)) antagonist (at a dose that lowers systemic blood pressure to a comparable extent) were equally effective in preventing urinary proteins in rats genetically predisposed to proteinuria and progressive renal dysfunction (37, 38). These studies represent experimental proofs that the ACE inhibitors' beneficial effects of preserving glomerular permselective function in many models of renal disease (1, 3, 40, 54–56) and protecting from progressive damage are essentially mediated by blocking the formation of ANG II rather than by other hormonal systems simultaneously activated by this class of drugs.

The results of previous investigations and of the study presented here allow some interesting observations on the relation between antihypertensive and antiproteinuric effects of either drug (or class of compounds). It has been suggested that the beneficial antiproteinuric effect of ANG II antagonism may be dissociated with the antihypertensive action of these therapies (16, 21, 41). Our present results would suggest that, on the acute and chronic level, there is no correlation between the antihypertensive effects of enalapril and irbesartan and changes in glomerular hemodynamics and urinary protein excretion (see Figs. 1 and 4). These results would further support the already postulated notion that the antiproteinuric effect of ANG II antagonism is nonhemodynamic, depending instead on the beneficial effect of reducing this hormone's biological activity on cellular functions that, at the glomerular capillary wall, result in amelioration of the permselective function.

A growing body of evidence is available that indicates greater proteinuria is associated with a faster GFR decline (47) and that reduction in urinary proteins, independent of the reduction in blood pressure, is associated with a subsequent beneficial effect on the progression of renal disease (34). These findings corroborate the hypothesis that enhanced protein traffic is not only a risk factor for faster GFR decline but may also contribute pathogenically to progression of renal disease (43). Controlled trials demonstrating that, for comparable blood pressure reductions, agents that more effectively decrease proteinuria are more renoprotective in the long term (19) strongly suggest that antihypertensive therapy should not simply be targeted to reduce blood pressure but possibly and more importantly to minimize proteinuria as well. Furthermore, this evidence and the present results indicate that minimizing blood pressure cannot necessarily be
the only or the ideal sensitive target of future renoprotective strategies.

In summary, our investigation documented that ACE inhibition and ANG II receptor antagonism comparably ameliorated urinary protein excretion and glomerular size-selective function in IgA nephropathy. The beneficial effects of the two drugs tested are not directly related to changes in blood pressure and suggest that ANG II plays a key role in the mechanism responsible for the development of glomerular membrane dysfunction in this renal disease.

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