Maximal suppression of renin-angiotensin system in nonproliferative glomerulonephritis

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Background. Elimination of residual proteinuria is the novel target in renoprotection; nevertheless, whether a greater suppression of renin-angiotensin system (RAS) effectively improves the antiproteinuric response in patients with moderate proteinuria remains ill-defined.

Methods. We evaluated the effects of maximizing RAS suppression on quantitative and qualitative proteinuria in ten patients with stable nonnephrotic proteinuria (2.55 ± 0.94 g/24 hours) due to primary nonproliferative glomerulonephritis (NPGN), and normal values of creatinine clearance (103 ± 17 mL/min). The study was divided in three consecutive phases: (1) four subsequent 1-month periods of ramipril at the dose of 2.5, 5.0, 10, and 20 mg/day; (2) 2 months of ramipril 20 mg/day + irbesartan 300 mg/day; and (3) 2 months of irbesartan 300 mg/day alone.

Results. Maximizing RAS suppression was not coupled with any major effect on renal function and blood pressure; conversely, a significant decrement in hemoglobin levels, of 0.8 g/dL on average, was observed during up-titration of ramipril dose. The 2.5 mg dose of ramipril significantly decreased proteinuria by 29%. Similar changes were detected after irbesartan alone (−28%). The antiproteinuric effect was not improved either by the higher ramipril doses (−30% after the 20 mg dose) or after combined treatment (−33%). The reduction of proteinuria led to amelioration of the markers of tubular damage, as testified by the significant decrement of α1 microglobulin (α1m) excretion and of the tubular component of proteinuria at sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Conclusion. In nonnephrotic NPGN patients, standard doses of either ramipril or irbesartan lead to significant reduction of residual proteinuria and amelioration of the qualitative features suggestive of tubular damage. The enhancement of RAS suppression up to the maximal degree does not improve the antiproteinuric response and is coupled with a decrement of hemoglobin levels.

Proteinuria is an important and independent risk factor for disease progression and end-stage renal disease (ESRD) in nondiabetic chronic nephropathies [1]; protein filtration is, in fact, associated with protein overabsorption by renal tubules that induces inflammatory events and, ultimately, damage at the tubulointerstitial level [2, 3]. In nonproliferative glomerulonephritis (NPGN), the contribution of proteinuria to the pathophysiology of progression of renal damage is more prominent than in proliferative glomerulopathies, where other factors play a role [2]. Identification of the most effective antiproteinuric treatment becomes therefore critical in NPGN.

In NPGN, the presence of nephrotic syndrome identifies the best candidates to immunosuppressive treatment; however, this therapeutic intervention allows a complete remission of proteinuria in about 50% of cases [4–6]. In addition, moderate degrees of proteinuria are detected since the beginning of the disease or during relapses in a substantial number of patients [4–6]. In these patients, with either induced or spontaneous residual proteinuria, treatment remains ill-defined. Conversely, it is important to underscore that long-term studies have demonstrated that, in NPGN, only complete remission of proteinuria ensures excellent renal prognosis [5, 6], while persistence of even moderate degrees of proteinuria is still coupled with progressive decline of renal function and, moreover, with increased risk for cardiovascular mortality [7, 8]. Indeed, elimination of residual proteinuria is now considered as the main target in renoprotection [9, 10]. To this aim, it has been proposed to up-titrate the dose of agents interfering with the renin-angiotensin system (RAS) to antiproteinuric rather than antihypertensive effect and.

Key words: membranous nephropathy, focal segmental glomerulosclerosis, converting enzyme inhibitor, angiotensin receptor antagonist, SDS-PAGE, urinary α1m.
to start such a treatment early in the course of disease, that is, prior to development of chronic renal failure [9–11].

In this regard, we have recently evidenced in a small group of patients with moderate proteinuria due to proliferative glomerulonephritis (IgA nephropathy) that co-administration of angiotensin-converting enzyme inhibitor (ACE-I) and angiotensin II (Ang II) AT₁ receptor antagonist (ARA) decreases proteinuria by a greater extent (−63%) than the single agents alone (−35%) [12, 13]. The rationale for combination therapy is based on the assumption that ARA would counteract the AT₁-mediated effects of Ang II formation by non-ACE enzymes while ACE-I would additionally increase kinins [14]. Whether enhancement of RAS suppression allows a major antiproteinuric response in NPGN patients with moderate proteinuria is unknown.

In the present work, we studied NPGN patients with nonnephrotic proteinuria and normal glomerular filtration rate (GFR). We monitored the antiproteinuric response during treatment with ramipril at doses progressively increased in a stepwise fashion, from 2.5 up to 20 mg/day, then after adding irbesartan 300 mg/day to the maximal dose of ACE-I, and, finally, after irbesartan alone. The study was aimed at evaluating whether in these patients the antiproteinuric response to ACE-I is a dose-related phenomenon and add-on ARA enhances the antiproteinuric effect of maximized ACE inhibition. Since, in NPGN, renal prognosis is significantly worsened by the urinary excretion of proteins of low molecular weight [15–17], the so-called tubular proteinuria, we also evaluated the effect of maximal RAS suppression on the qualitative features of proteinuria.

METHODS

Patients

We screened all adult patients followed in the outpatient renal clinic from December 1998 through December 2000 to select those who met the following enrollment criteria: biopsy-proven primary NPGN, moderate proteinuria (1.0 to 3.5 g/day), and serum albumin levels ≥3.0 g/dL persisting for at least 12 months prior to the study in the absence of any immunosuppressive treatment and normal renal function (creatinine clearance >70 mL/min). We excluded patients with evidence of edema, diabetes mellitus, renovascular disease, obstructive uropathy, serum potassium ≥5.5 mmol/L, cancer, chronic treatment with anti-inflammatory drugs, and known or suspected intolerance to ACE-I or ARA.

Ten patients out of the 31 patients were found to be eligible in the 2-year period of screening and gave their informed consent to enter onto the study. The remaining 21 eligible patients refused enrollment. The reasons they gave included their fear of the potential side effects of the high-dose “antihypertensive” therapy or because of the large number of visits and/or excessive distance of the hospital from their residence.

Study design

This was an open-label study, performed on ambulatory basis, designed to compare prospectively the antiproteinuric response to stepwise enhancement of RAS suppression with the patients’ basal values as their own control. The study lasted 14 months (Fig. 1). Two months after withdrawal of eventual therapy with ACE-I or ARA (run in), patients underwent the clinical and biochemical basal evaluation and started the active treatment protocol constituted by three consecutive phases: (1) four subsequent 1-month periods of treatment with ramipril at the dose of 2.5, 5.0, 10, and 20 mg/day; (2) 2 months of ramipril 20 mg/day + irbesartan 300 mg/day; and (3) 2 months of irbesartan 300 mg/day alone. Two-month intervals of washout followed each phase. Study medication was administered twice a day (8:00 a.m. and 8:00 p.m.); in the study days, the morning dose was taken after data collection at the outpatient clinic (between 8:00 and 9:00 a.m.). All patients were required to maintain their usual salt intake throughout the period of the study.

Clinical and laboratory procedures

Assessment of clinical and laboratory parameters was performed at baseline, at the end of each month of the first phase, at the end of the subsequent two phases, and at the end of each of the three washout periods. In addition, to ensure the safety of the patients, serum creatinine and potassium, as well as blood pressure, were measured between the 10th and the 15th day following any change of medication prescription. We measured at each step proteinuria, urinary levels of α₁-microglobulin (α₁,m), creatinine, sodium (Uₜₘₜ), and urea, as well as body weight, blood pressure, serum creatinine, potassium, albumin, hemoglobin, peripheral plasma renin activity (PRA), and aldosterone.

Blood pressure was measured in the morning before drug administration by a mercury sphygmomanometer after 10 minutes’ rest. The first and fifth Korotkoff sounds were used as systolic and diastolic blood pressure levels.
The mean of three consecutive measurements taken 2 minutes apart in the sitting position was used for statistical analysis. Mean blood pressure was calculated as the sum of one third of systolic and two thirds of diastolic blood pressure. Blood pressure measurements were repeated after 5 minutes in standing position to detect eventual orthostatic hypotension.

All the urinary samples were stored and analyzed at the same time. At each step, proteinuria was measured in two consecutive 24-hour urine collections (the mean value was recorded and used for statistical analysis) with the pyrogallol red-molibdate method; the intra-assay and interassay coefficients of variation of this method was less than 2%. The urinary levels of α1m were assessed by immunonephelometric method on a Coulter Beckman nephelometer, using caprine serum antihuman α1m (Beckman, Fullerton, CA, USA). The intra-assay and inter-assay coefficients of variation of this method was less than 5%.

The pattern of urinary proteins was determined at baseline, and at the end of the three phases (up titration of ramipril dose, combined treatment, and irbesartan alone) by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), as previously described [15]. The Mini-Protean II apparatus (Bio-Rad Laboratories, Hercules, CA, USA) was used. Urine samples (the volume ranged from 2 to 19 μL to apply a protein amount of 3.5 μg) were applied to polyacrylamide gel slab (85 × 70 × 1 mm, 12% of acrylamide/N,N'-methylene bis-acrylamide, 30% weight/volume). Electrophoresis was programmed to run at 60 volts for stacking and 110 volts for running layer of slabs in discontinuous running buffer, using the Bio-Rad Power Supply Model 200/2.0 apparatus (Bio-Rad Laboratories). The gels were stained with Coomassie blue R 250 (which produces blue protein bands with sensitivity as low as 8 to 28 ng) for 30 minutes at room temperature; subsequently, they were destained with an acetic acid/methanol/aqueous solution (1:5:4 volume) to give sufficiently clear background and fixed between two cellophane papers and dried. The molecular weight of urinary protein bands was assessed by comparison with standard protein of known molecular weight (10 kD Protein Ladder; GIBCO BRL, Uxbridge, UK), using Ultrascan XL 2222 Densitometer (Pharmacia LKB Biotechnology AB, Uppsala, Sweden). The SDS-PAGE patterns are classified according to the presence of protein bands of different molecular weights [15, 16]. Specifically, the pattern characterized by the albumin band alone (68 kD) is considered “physiologic;” while the presence, beside albumin, of protein bands of molecular weight >68 kD identifies the “pure glomerular” pattern. A “tubular” pattern is defined by the presence, beside albumin and proteins with molecular weight >68, of protein bands with molecular weight <68 kD; specifically, two tubular patterns can be distinguished: the low-molecular-weight (LMW) and the very low-molecular-weight (VLMW) on the basis of the absence or presence of protein bands with a molecular weight <23 kD, respectively. For the analysis of SDS-PAGE data, we assigned a score to each pattern: VLMW = 1, LMW = 2, pure glomerular = 3, physiologic = 4; indeed, previous studies have demonstrated significant differences in terms of tubulointerstitial damage and renal prognosis among the four categories, with VLMW being characterized by the worst and physiologic by the best outcome [15, 16].

Serum albumin, serum and urinary levels of creatinine, urea, sodium, and potassium were measured by an auto-analyzer (Olympus AU 400, Olympus Italia, Segrate, Italy). Daily urea excretion was used to calculate protein intake as described in a previous paper by our group [18]. Hemoglobin was measured by Coulter counter (Coulter Electric, Hialeah, FL, USA). PRA and aldosterone were measured by a radioimmunoassay (RIA) technique using commercial kits (DPC, Los Angeles, CA, USA).

Statistics

Results are reported as mean ± SD. Analysis of variance for repeated measures was always applied to compare arms, using pretreatment values as covariates when appropriate. With only two groups to be compared that analysis reduces to a paired t test. Focus was on the following predefined questions: (1) Do the three different treatments (ACE-I, ARA, ACE-I + ARA) have any effect on pre-treatment values? (2) Is there a dose effect of ACE-I treatment? (3) Is the effect of ACE-I + ARA greater than that of single agents? Since treatments were assigned sequentially and a carryover bias could possibly confound results, a washout period of 2 months was planned among the different treatments; therefore, similarity of pretreatment conditions was preliminarily tested to exclude a carryover effect. A period effect could not be tested; indeed, we could not randomize the type of treatment because administration of either ACE-I at high dose or combined treatment without preliminary evaluation of the effects of ACE-I at lower doses would have been unethical in our normotensive patients. Finally, to test whether the antiproteinuric effect of treatment was mediated by the blood pressure lowering effect, the pre- and posttreatment values of proteinuria were analyzed using the respective values of blood pressure (systolic, diastolic, and mean) as covariates. We also estimated correlation coefficients between changes in proteinuria and concomitant changes in systolic blood pressure, diastolic blood pressure and mean blood pressure. Two-tailed significance level was 0.05.

A sample size of ten patients adequately allowed an average power of 89% (range, 80% to 95% depending on the number of groups compared) to detect a difference in means across the levels of repeated measures factor equal to half of the common standard deviation, that is,
an effect size of 0.5 at a significance level of 0.05 (nQuery Advisor 1.0, 1995).

RESULTS

We studied two females and eight males with a mean age of 34 years old (range, 21 to 51 years). The histologic diagnosis was idiopathic membranous nephropathy (N = 5) and primary focal segmental glomerulosclerosis (N = 5). Seven of the patients studied had been previously treated with immunosuppressive therapy (steroids alone or in combination with cyclophosphamide). At baseline, body weight was 69.3 ± 10 kg and serum albumin was 3.8 ± 0.6 g/dL; these values did not change throughout the different steps. Similarly, protein intake, that averaged 1.14 ± 0.12 g/kg body weight/day at baseline, did not vary subsequently.

We did not detect in any patient, at any step, either acute renal failure (that is, an increment of serum creatinine >30% versus pretreatment values), or hyperkalemia (that is, serum K ≥5.5 mmol/L). No symptomatic hypotensive episodes, dizziness, or fatigue were observed throughout the study.

The main clinical and biochemical features of patients according to the type of treatment are summarized in Table 1, with the exception of proteinuria and αm excretion that have been reported in Figure 2.

Carryover effect

No difference was found across the pretreatment values (baseline and washout periods) of the parameters examined, testifying that basal conditions were always restored after washout periods.

Effect of ACE-I treatment

No difference was detected for creatinine clearance, serum potassium, and UNaV, while a significant effect of ACE-I versus pretreatment conditions was observed for systolic blood pressure (P = 0.0001), diastolic blood pressure (P = 0.0007), mean blood pressure (P = 0.0002), PRA (P = 0.001), aldosterone (P = 0.04), and hemoglobin (P = 0.05).

ACE-I treatment significantly affected proteinuria (P = 0.0005) and αm (P = 0.03). At baseline, proteinuria averaged 2.55 ± 0.94 g/day (range, 1.1 to 3.2 g/day). The decrement of proteinuria during ACE-I was equal to 30% on average, with the lowest dose of ramipril (2.5 mg/day) inducing a 29% reduction.

Dose-effect of ACE-I

A statistically significant effect of the different doses of ramipril (from 2.5 up to 20 mg/day) was observed for PRA that increased from 4.2 to 7.1 ng/mL/hour (P = 0.004), aldosterone that decreased from 80 to 44 pg/mL (P = 0.006), αm that decreased from 26.0 to 19.2 mg/day (P = 0.04), and hemoglobin that decreased from 14.6 to 13.8 g/dL (P = 0.003). Conversely, no difference among the different doses of ACE-I was observed for proteinuria (P = 0.94), systolic blood pressure (P = 0.72), diastolic blood pressure (P = 0.62), and mean blood pressure (P = 0.64). The same held true for creatinine clearance, serum potassium, and UNaV.

Effect of ARA treatment

No difference was observed for creatinine clearance, serum potassium, UNaV, aldosterone, and hemoglobin, while a significant effect of ARA versus pretreatment conditions was observed for Uprot (P = 0.003) that decreased by 28%, αm (P = 0.04), systolic blood pressure (P = 0.003), diastolic blood pressure (P = 0.01), mean blood pressure (P = 0.003), and PRA (P = 0.0005).

Effect of combination ACE-I + ARA

A significant effect of combination versus pretreatment conditions was observed for proteinuria (P = 0.0009),
Effect of the combination ACE-I + ARA versus single agents

A significant difference among the three arms was observed only for PRA \((P = 0.01)\), aldosterone \((P = 0.005)\), and hemoglobin \((P = 0.02)\). Conversely, no difference was noted for proteinuria \((P = 0.94)\), that decreased by 33% in the combination arm, \(\alpha_m\) \((P = 0.33)\), systolic blood pressure \((P = 0.46)\), diastolic blood pressure \((P = 0.80)\), and mean blood pressure \((P = 0.62)\). The same held true for serum potassium, creatinine clearance, and \(U_{NaV}\).

Table 2. Pattern of urinary proteins at baseline and after up titration of ramipril dose [angiotensin-converting enzyme inhibitor (ACE-I)], combined treatment [ACE-I + angiotensin II AT1 receptor (ARA)] and irbesartan alone (ARA) at sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>ACE-I</th>
<th>ACE-I + ARA</th>
<th>ARA</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLMW number</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>LMW number</td>
<td>6</td>
<td>8</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>GLOM number</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>PHYS number</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Score</td>
<td>1.60 (\pm) 0.52</td>
<td>2.10 (\pm) 0.74*</td>
<td>2.10 (\pm) 0.74*</td>
<td>2.20 (\pm) 0.79*</td>
</tr>
</tbody>
</table>

The values are mean \(\pm\) SD. Abbreviations are: VLMW, very low-molecular-weight pattern; LMW, low-molecular-weight pattern; GLOM, glomerular pattern; PHYS, physiologic pattern. The score assigned at each pattern was as following: VLMW = 1, LMW = 2, GLOM = 3, PHYS = 4.

No further change was detected in the subsequent steps with the exception of a shift from LMW to pure glomerular pattern in one patient at the end of the study. The amelioration of pattern was testified by the significant increase of score.

Relationship between antiproteinuric and blood pressure lowering effect

After adjustment by values of blood pressure (systolic, diastolic, and mean), the mean values of proteinuria did not significantly vary during ramipril 2.5 mg/day (from an unadjusted decrease of 0.8 g/day to values of 0.7 g/day adjusted for systolic, diastolic, and mean blood pressures). Similar results were obtained when considering the higher doses of ramipril, combined treatment, and irbesartan alone. Accordingly, no significant correlation was found between the percentual changes of proteinuria and systolic, diastolic, and mean blood pressures.

Effects of treatments on pattern of proteinuria

In basal conditions, all the patients showed a tubular pattern of proteinuria at SDS-PAGE analysis; in particular, the pattern was LMW in six and VLMW in four patients (Table 2). At the end of up-titration of ramipril dose, the pattern changed from VLMW to LMW in three patients, and from LMW to physiologic in one patient. No further change was detected in the subsequent steps with the exception of a shift from LMW to pure glomerular pattern in one patient at the end of the study. The amelioration of pattern was testified by the significant increase of score.

DISCUSSION

In NPGN, previous studies have evaluated the antiproteinuric effect of either increasing the dosage of ACE-I/ARA [19–23], or combining the two agents [23, 24]. In all these studies, however, patients were characterized by nephrotic proteinuria and renal dysfunction, that is, features that usually indicate prescription of immunosuppressive treatment; a reduction of proteinuria below the nephrotic range was in fact hardly achieved. The current work is the first study that addresses the question as whether enhancement of RAS suppression amelio-
rates the antiproteinuric response in NPGN patients with moderate proteinuria and preserved renal function that are usually treated with symptomatic therapy. Suppression of RAS was pushed up to the maximal degree by stepwise increase of ramipril dosage from 2.5 up to 20 mg/day, that is, a dose four-fold greater than the maximal dosage of the REIN study [11], followed by add-on treatment with 300 mg/day of irbesartan, that is, the maximal dose used currently in clinical trials [25]. This two-phase analysis is essential to verify the presence of additive effects of the combination therapy since both agents act of the same system. Indeed, Agarwal [26] has recently demonstrated in diabetic patients with chronic renal failure the absence of any specific advantage of adding ARA on top of ACE-I when the latter is administered at high dose. Conversely, in most of the studies published to date on the antiproteinuric effect of combined therapy in both proliferative and NPGN, the impact of ACE-I + ARA has been tested without preliminary evaluation of the effects of ACE-I at doses higher than usual.

All the ramipril doses administered and the combination therapy were well tolerated and not associated with significant side effects. Of note, however, hemoglobin levels tended to diminish when ramipril dose was increased, with the maximal reduction of 0.8 g/dL on average, detected after administration of the 20 mg dose. On the contrary, no hemoglobin change was noted after administration of irbesartan alone. The class-specific lowering effect of ACE-I on hemoglobin levels, recently reported also in renal transplant recipients [27], has been ascribed to the reduced degradation of a physiologic inhibitor of erythropoiesis [28]. The clinical implication of these data may not be trivial because even a 0.5 g/dL decrement of hemoglobin levels is associated with significant growth of left ventricular mass in patients with anemia secondary to chronic renal failure [29].

Ramipril induced a reduction of proteinuria, of about 30% on average (Fig. 2), in the absence of changes of creatinine clearance and salt intake. The 2.5 mg/day dose of ramipril was sufficient to determine this effect, and a similar reduction was observed after irbesartan alone. Restoration of proteinuria to basal values in the washout periods demonstrates that the positive results on proteinuria were not achieved by chance or dependent on spontaneous remission of underlying disease. The extent of the antiproteinuric response to the usual doses of ACE-I or ARA is analogous to that we have previously observed in a small group of patients with similar characteristics (moderate proteinuria, normal GFR, and blood pressure) but with IgA nephropathy [12, 13].

However, at variance with our previous studies [12, 13], in the current work no improvement of the antiproteinuric effect was evidenced after enhancing RAS suppression; in fact, neither the increase of ramipril dose nor add-on ARA led to additional decrement of proteinuria (Fig. 2), despite the presence of a stepwise elevation in PRA values. Of note, the results of the current study actually differ from what has been reported in nondiabetic glomerulopathies characterized by expansion of mesangial area. Similarly to IgA nephropathy, in fact, also in NPGN patients with nephrotic syndrome either the increase of the dose of ACE-I/ARA or the coadministration of the two agents allow a greater decrement of proteinuria [19–24]. On the basis of these findings, it is reasonable to hypothesize that after enhancing RAS suppression a larger antiproteinuric effect may become manifest only in the presence of significant mesangial expansion secondary to mesangium overload, as in the presence of severe proteinuria, or primarily caused by the underlying disease, as in the case of IgA nephropathy. Indeed, proliferation of mesangial cells is associated with high sensitivity to the beneficial effects of ACE-I and ARA on glomerular structure and function [30]; on the other hand, the glomerular response to Ang II disappears after mesangial cell lysis [31]. This hypothesis may therefore account for the lack of a greater antiproteinuric effect in response to maximized RAS suppression in our NPGN patients with moderate proteinuria.

In our patients, we had to maintain a normal salt intake because of the optimal blood pressure levels detected prior to the study; consequently, we cannot exclude that a larger antiproteinuric effect of maximal RAS suppression would have been attained at low sodium diet [19, 23]. Furthermore, this trial was designed to assess the short-term antiproteinuric response to a stepwise enhancement of RAS suppression; therefore, we cannot exclude that the higher doses of ramipril or add-on ARA treatment may allow a more favorable outcome of renal function in the long-term. Nevertheless, the short-term antiproteinuric response achieved by the low doses of ACE-I or ARA can be reasonably considered as already predictive of a more benign course [1, 9–11].

Our clinical study was not primarily designed to investigate on the mechanism(s) of the antiproteinuric response to RAS suppression; indeed, previous studies in nondiabetic patients with nephrotic syndrome have addressed this issue, showing that either standard or higher ACE-I doses induce a specific improvement of glomerular barrier size-selective dysfunction independently from the antihypertensive effect [20, 22, 32]. In the present work, blood pressure decreased of about 10% after the 2.5 mg dose of ramipril, and, by the same extent, after irbesartan with no further decrement in response to higher ACE-I doses or to add-on ARA. Nevertheless, analysis of covariance and the absence of any correlation between the changes of proteinuria and of blood pressure suggest that the decrease of proteinuria was largely independent from the changes of systemic blood pressure.

Further novel insights into the antiproteinuric response to RAS suppression were obtained when evaluating the
qualitative figures of proteinuria. In glomerulonephritic patients, presence of tubular proteinuria correlates with the progressive impairment of GFR better than the absolute amount of total proteinuria, with the worst outcome being associated with the presence of proteins of very low molecular weight [15, 16]. At baseline, all the patients were characterized by the presence of a tubular pattern at the SDS-PAGE analysis of proteinuria. In addition, we also found an elevated excretion of $\alpha_1$m that is considered as a marker of established tubular damage; under normal conditions, in fact, this protein of low molecular weight does not appear in the urine because it is freely filtered through the glomerular capillary membrane and completely reassorbed by the tubular cells. Bazzi et al [17] have demonstrated that a high $\alpha_1$m excretion predicts the progression of membranous nephropathy to renal failure better than total proteinuria. In our study, during treatment, urinary $\alpha_1$m excretion decreased and a less severe tubular component of proteinuria became evident at SDS-PAGE analysis (Table 2). Both the changes of $\alpha_1$m excretion and the shift of SDS-PAGE pattern are compatible with the hypothesis that the reduction of glomerular loss of proteins attenuates the reversible events of the proteinuria-induced injury at the tubular level, therefore improving the tubular reabsorption of the low-molecular-weight proteins [2, 3].

CONCLUSION

In nonnephrotic NPGN patients with normal renal function (1) administration of standard doses of either ramipril or irbesartan significantly decreases, by about 30%, the extent of proteinuria; (2) the antiproteinuric effect is associated with amelioration of qualitative features of proteinuria suggestive of tubular damage; and (3) increasing RAS suppression up to the maximal degree does not improve the antiproteinuric response and is possibly coupled with a significant decrement of hemoglobin levels. This study therefore suggests that maximal RAS suppression does not consistently allow a major antiproteinuric effect in proteinuric patients; further studies are required to substantiate the hypothesis that the magnitude of response depends on the degree of activation of mesangium.

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REFERENCES

5. LALUCK BJ, CATTRAN DC: Prognosis after a complete remission in adult patients with idiopathic membranous nephropathy. Am J Kidney Dis 33:1026–1032, 1999


