Additive Antiproteinuric Effect of Converting Enzyme Inhibition and a Low Protein Intake¹

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ABSTRACT
The hypothesis that converting enzyme inhibition and a protein-restricted diet could have additive antiproteinuric effects has been tested. A group of 17 patients with proteinuria in excess of 3 g/24 h per 1.73 m² of body surface area were submitted to a 3-wk period of study, after a 4-wk wash-out period during which protein intake was 1.0 g/kg per day and in the absence of any medication. During the first and second weeks of the study, protein intake was lowered to 0.3 g/kg per day, and in the third week, it returned to 1.0 g/kg per day. Enalapril (20 mg daily) was administered during the second and third weeks of the study. Initially and at the end of each week thereafter, we determined blood pressure, GFR (insulin clearance), RPF (para-aminohippurate clearance), plasma sodium and potassium, PRA and aldosterone, and the 24-h urine excretion of sodium, potassium, protein, and urea. The low protein intake during the first week induced a significant fall of proteinuria (P < 0.01), GFR (P < 0.01), and RPF (P < 0.01) in the absence of changes in filtration fraction. The addition of enalapril induced a further decrease of proteinuria (P < 0.01) and a fall in filtration fraction (P < 0.05), whereas plasma potassium, PRA, GFR, and RPF values increased (P < 0.01). The rise in protein intake during the last week of the study induced a significant rise in proteinuria, GFR, and RPF (P < 0.01), although the first of these parameters attained values significantly lower (P < 0.05) than those observed initially. These results indicate that a low protein intake and converting enzyme inhibition have an additive antiproteinuric effect in the presence of a reversal of the fall in GFR and RPF induced by the diet.

Key Words: Proteinuria, enalapril, renal hemodynamics, PRA, aldosterone

Protein intake has a striking effect on urinary protein excretion in normal humans (1). A low protein intake has been shown to decrease the rate of proteinuria in patients with chronic renal diseases (2-4) and has been advocated as a means to slow the progression of chronic renal failure (5). Additionally, a low-protein diet induces a fall of RBF and GFR in the absence of changes in filtration fraction (1, 6).

On the other hand, angiotensin-converting enzyme inhibition has also been shown to reduce proteinuria of glomerular origin in humans while inducing an increase in RPF, which may be accompanied by a fall in GFR (7, 8).

Both a low protein intake (4) and converting enzyme inhibition (9) improve the size-selective defect in glomerular permeability. Nevertheless, their effects on the renin-angiotensin system seem to be the opposite with an increase in the production of or in the sensitivity to angiotensin II in the case of the low protein intake (10) and with an inhibition in the synthesis of this peptide when angiotensin-converting enzyme inhibitors are used (11). We have hypothesized that in humans presenting with nondiabetic glomerular nephrotic proteinuria, there could be a synergistic antiproteinuric effect with the simultaneous usage of a low protein intake and of a converting enzyme inhibitor.

METHODS
Subjects
A group of 17 patients presenting with 24-h proteinuria within the nephrotic range (>3 g/24 h per 1.73 m² body surface area) and normoalbuminemia (12) was included in the study. Ten were men and seven were women with ages ranging from 20 to 64 yr and creatinine clearance between 33 and 146 mL/min.

They had been previously diagnosed as having reflux nephropathy (N = 6), immunoglobulin nephropathy (N = 4), focal and segmental glomerulosclerosis (N = 3), membranoproliferative glomerulonephritis (N = 2), membranous glomerulonephritis (N = 1), and...
Proteinuria, Converting Enzyme Inhibition, and Protein Intake

Alport syndrome (N = 1). The diagnosis was biopsy proven in the cases with immunoglobulin A nephropathy, focal and segmental glomerulosclerosis, membranoproliferative and membranous glomerulonephritis, and Alport syndrome. The diagnosis of reflux nephropathy was based on the radiologic finding of calyceal abnormalities and parenchymal loss at iv urography accompanied by bilateral vesicoureteral reflux at micturating cystography. Arterial hypertension (blood pressure in excess of 140/90 mm Hg) was initially present in nine. In all of them, therapy with a converting enzyme inhibitor had been previously maintained for at least 1 month and had been shown to induce a decrease of 24-h proteinuria of more than 30% of the basal value.

Study Design

After a wash-out period of 4 wk during which therapy with the converting enzyme inhibitor was withdrawn and protein intake was maintained at 1 g/kg per day, patients were submitted to a 3-wk period of study. During the first and second weeks, protein intake was decreased to 0.3 g/kg per day, and in the third week, it was increased to 1 g/kg per day. Both diets were isocaloric, with the protein content in the low-protein diet coming half from animal and half from vegetable sources and with around 60% of the energy from carbohydrate and around 30% from fat. Salt Intake was unrestricted, and the potassium content of each diet was 60 mmol/day. Each individual's food intake was assessed by a diet history. Enalapril (20 mg every day) was administered during the second and third weeks. At the end of the wash-out period and once weekly thereafter, the following parameters were determined: blood pressure, heart rate, body weight, GFR (inulin clearance), RPF (parainomohippurate [PAH] clearance), serum sodium and potassium, PRA and aldosterone, and 24-h urinary excretion of sodium, potassium, protein, and urea.

Experiments were performed at the same time of the day. On the morning of the test, the patients were admitted, fasting, to a Metabolic Ward. A teflon cannula was inserted into the antecubital vein of each arm for infusion and blood sampling, respectively. An injection of bolus doses of inulin and PAH (50 and 8 mg/kg, respectively) were administered, followed by a continuous infusion at rates of 34 mg/kg per h for inulin and 13.6 mg/kg per h for PAH in isotonic saline. After 45 min of equilibration, three timed (30-min) urine collections were made. At the midpoint of each urine collection period, blood samples were drawn. The concentrations of inulin and PAH were estimated by photocolorimetric methods. Blood pressure was measured every 15 min with an automatic recorder (Dynamap Model 845; Critikon, Inc., FL). Values of this parameter are expressed as the mean of the first four measurements. Laboratory procedures have previously been described (13–15).

An informed consent was obtained from every patient, and the protocol was approved by the Ethics Committee of the 12 de Octubre Hospital.

Statistical Analysis

Values are expressed as mean ± standard error (SE). Analysis of the data was performed by nonparametric tests, with Friedman's analysis of variance by ranks to identify global differences between treatments and the Wilcoxon's signed rank test for paired comparisons between the different parts of the study. Statistical significance was assumed when the P value was less than 5%.

RESULTS

As can be seen in Tables 1 and 2 blood pressure did not change when protein intake was diminished and fell significantly (P < 0.01) when enalapril was administered in a fashion independent of the protein intake. Meanwhile, proteinuria fell when protein intake was reduced (P < 0.01); enalapril induced a further decrease of this parameter (P < 0.01) but was unable to prevent an increase of this parameter when protein intake returned to normal values (P < 0.01), although the levels were lower than those observed initially (P < 0.05). Table 1 and 2 also shows how GFR and RPF decreased with the low protein intake (P < 0.01) and filtration fraction remained stable. The addition of the converting enzyme inhibitor induced an augmentation of both parameters (P < 0.01) and a decrease of filtration fraction (P < 0.05). With the shift of protein intake to normal, GFR and RPF went up again (P < 0.01) and filtration fraction remained in values lower than the initial values (P < 0.05). No correlation was found for proteinuria with GFR or RPF, but a significant correlation was found between this parameter and both systolic (r = 0.5483; P < 0.001) and diastolic blood pressure (r = 0.3752; P < 0.01).

PRA exhibited the expected increase after converting enzyme inhibition (P < 0.01), accompanied by a significant fall of plasma aldosterone (P < 0.01). Plasma sodium did not change and plasma potassium increased significantly when protein intake was decreased (P < 0.05) and even more when enalapril was administered (P < 0.01).

A significant fall in the urine excretion of urea was observed when protein intake was decreased (P < 0.01), in the absence of differences between the first and the last stages of the study. No change was observed in the urinary excretion of sodium or potassium.
TABLE 1. Values of blood pressure, 24-h proteinuria, GFR, RPF, filtration fraction, PRA, plasma aldosterone, plasma sodium and potassium, and urine excretion of sodium, potassium, and urea in the four stages of the study

<table>
<thead>
<tr>
<th>Initial</th>
<th>LPI</th>
<th>LPI + E</th>
<th>NPI + E</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mm Hg)</td>
<td>132.5 ± 3.9</td>
<td>130.1 ± 4.4</td>
<td>121.5 ± 2.8</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>85.5 ± 2.2</td>
<td>85.4 ± 2.3</td>
<td>78.5 ± 1.8</td>
</tr>
<tr>
<td>Proteinuria (g/24 h)</td>
<td>3.81 ± 0.44</td>
<td>2.59 ± 0.38</td>
<td>1.71 ± 0.37</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
<td>85.8 ± 8.8</td>
<td>61.2 ± 6.8</td>
<td>76.0 ± 8.7</td>
</tr>
<tr>
<td>RPF (ml/min)</td>
<td>400.9 ± 38.4</td>
<td>334.0 ± 33.5</td>
<td>388.0 ± 39.4</td>
</tr>
<tr>
<td>FF</td>
<td>0.22 ± 0.03</td>
<td>0.19 ± 0.04</td>
<td>0.18 ± 0.03</td>
</tr>
<tr>
<td>PRA (ng/L-1/s)</td>
<td>0.80 ± 0.13</td>
<td>0.86 ± 0.21</td>
<td>2.44 ± 0.30</td>
</tr>
<tr>
<td>PA (mmol/L)</td>
<td>0.33 ± 0.04</td>
<td>0.43 ± 0.06</td>
<td>0.27 ± 0.04</td>
</tr>
<tr>
<td>Kp (mmol/L)</td>
<td>141.8 ± 0.5</td>
<td>141.8 ± 0.7</td>
<td>140.7 ± 0.54</td>
</tr>
<tr>
<td>NaU (mmol/24 h)</td>
<td>172.11 ± 19.10</td>
<td>124.70 ± 22.21</td>
<td>136.82 ± 16.04</td>
</tr>
<tr>
<td>KU (mmol/24 h)</td>
<td>70.17 ± 4.19</td>
<td>73.70 ± 6.74</td>
<td>66.17 ± 5.18</td>
</tr>
<tr>
<td>Urea (mmol/24 h)</td>
<td>925.7 ± 97.7</td>
<td>712.3 ± 79.9</td>
<td>750.2 ± 67.8</td>
</tr>
</tbody>
</table>

Abbreviations: FF, filtration fraction; PA, plasma aldosterone; Nap, plasma sodium; Kp, plasma potassium; NaU, urine excretion of sodium; KU, urine excretion of potassium; LPI, low protein intake; E, enalapril; NPI, normal protein intake; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 2. P values of comparisons of the various diets

<table>
<thead>
<tr>
<th>BP</th>
<th>Protein</th>
<th>GFR</th>
<th>RPF</th>
<th>FF</th>
<th>PRA</th>
<th>PA</th>
<th>Nap</th>
<th>Kp</th>
<th>NaU</th>
<th>KU</th>
<th>Urea</th>
</tr>
</thead>
<tbody>
<tr>
<td>I versus LPI</td>
<td>NS</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>I versus LPI + E</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>I versus NPI + E</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LPI versus NPI + E</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>LPI versus NPI + E</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
<td>NS</td>
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<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LPI + E versus NPI + E</td>
<td>NS</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.01</td>
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</tr>
</tbody>
</table>

Abbreviations: BP, blood pressure; FF, filtration fraction; PA, plasma aldosterone; Nap, plasma sodium; Kp, plasma potassium; NaU, urine excretion of sodium; KU, urine excretion of potassium; I, initial; LPI, low protein intake; E, enalapril; NPI, normal protein intake; NS, not significant.

**DISCUSSION**

The persistence of heavy proteinuria is associated with an increased risk of progression to end-stage renal failure. Recently, it has been stressed that proteinuria could be a marker of intraglomerular hemodynamic changes and could participate in the development of glomerulosclerosis and tubulointerstitial damage while facilitating the induction of hyperlipidemia that, in turn, can aggravate the renal damage (16). Hence, any reduction in urine protein excretion could be of value for arresting the progression of renal failure and should be regarded as a good prognostic index (2, 15, 17).

A protein-restricted diet has been shown to decrease the renal excretion of proteins in normal subjects as well as in patients presenting with glomerular proteinuria (1–4). Our results are in agreement with those reports, and the adequate performance of the diet was ensured by the changes in urine urea excretion. The mechanisms involved in the renal effects of protein restriction have been shown to be reductions in capillary plasma flow rate, in glomerular capillary pressure, and in ultrafiltration coefficient, the modification of glomerular eicosanoid metabolism with a reduction in prostaglandin E2 and thromboxane B2 levels, a diminished sensitivity to agonists, and the preservation of the glomerular capillary wall anionic charge (18, 19). Reports on the effect of protein restriction on GFR and RPF have shown a fall of both parameters in the experimental animal (10, 18), in normal humans (1, 20, 21), and in patients with different degrees of renal failure (22, 23). In our group of patients, both GFR and RPF fell significantly when protein intake was diminished and filtration fraction remained unchanged. We found no correlation between changes in GFR and changes in protein excretion, indicating, with some reserve, that the effect on this parameter is not primarily dependent on a change in the quantity of filtered proteins. An absence of changes in renal hemodynamics with protein restriction has neverthe-
less been shown, usually in patients presenting with moderate renal failure (4, 24, 25). In those studies, antihypertensive therapy was maintained and could have contributed to blunt the renal response to protein restriction, especially if calcium channel blockers, which can decrease the ability of the kidney to autoregulate, were being used (26), as in the studies of Don et al. (24) and Remuzzi et al. (25). The degree of renal insufficiency could also contribute to explain conflicting results. The protective effects of both dietary and nondietary interventions seem to be most effective when at least 50% of the residual renal mass is still functioning (27). Our group of patients presented with GFR levels clearly above those of the patients in studies by Rosenberg et al. (4) and Don et al. (24).

In contrast with previous results (4), our results did not indicate a fall in the components of the renin-angiotensin system measured. The explanation for the absence of a decrease in PRA could be because Rosenberg et al. (4) used a diet containing 2 g of protein per day in the high-protein-diet phase of their study, whereas in our study, we used a diet containing only 1 g/kg per day.

The administration of a converting enzyme inhibitor when protein intake was restricted resulted, in our hands, in a further fall of proteinuria that was accompanied by a reversal of the renal hemodynamic effect of the diet. Similar results have been described in rats (28). Both angiotensin II and a decreased synthesis of vasodilator prostaglandins have been shown to mediate the changes in intrarenal hemodynamics induced by protein deprivation in animals (6, 10, 29). In fact, Murray (30) has shown that, in rats, the vascular response to angiotensin II remains intact when proteins are restricted in the diet and that renal vasoconstriction observed in this situation appears to be mediated by angiotensin II. These findings can explain how the addition of a converting enzyme inhibitor to the low protein intake induces a further decrease in proteinuria, probably through a mechanism not very distant from the additive antiproteinuric effect of converting enzyme inhibition when sodium intake is reduced, as shown by Heeg et al. (31). Similarly, a reversal of the renal hemodynamic changes induced by such a diet can be expected with the simultaneous administration of a converting enzyme inhibitor as shown by Fernandez-Repollet and Tapia (10) in animals and by ourselves in humans. The fall in filtration fraction observed when enalapril was given to our patients indicates that a fall in efferent renal arteriolar resistance has probably taken place (7). The participation of a stimulated prostaglandin E2 and/or kallikrein-kinin by the action of enalapril (10, 32) on both renal hemodynamics and proteinuria cannot be discarded. The reintroduction of a normal protein intake was accompanied by a significant increase in proteinuria, GFR, and RPF, whereas while filtration fraction remained in values lower than those observed initially. The effect of a converting enzyme inhibitor on the renal response to an acute protein load has been shown to blunt the increase of both GFR and RPF induced by that maneuver in patients with chronic renal insufficiency (33) but not in patients with essential hypertension and normal GFR (34). These data could indicate that the presence or absence of a preserved renal function could modulate the effect of angiotensin-converting enzyme inhibition on the renal response to the protein content of the diet. The degree of the renal vasodilation induced by the diet was, nevertheless, blunt if we compare the increase with the change that took place when protein intake was reduced during the first week of the study. An increase in the protein content of the diet enhances the renal production of vasodilating agents such as prostaglandins (4, 6, 19, 32, 35), and the participation of this mechanism cannot be excluded to explain the renal vasodilation observed in our results. Meanwhile, proteinuria remained in values below those observed initially, indicating that angiotensin-converting enzyme inhibition was effective in reducing protein excretion.

In summary, this study shows that there is an additive antiproteinuric effect of a low protein intake and converting enzyme inhibition. The clinical relevance of this finding in the progression of chronic renal failure remains to be elucidated.

REFERENCES