Effects of UP269-6, a New Angiotensin II Receptor Antagonist, and Captopril on the Progression of Rat Diabetic Nephropathy

Eduardo Villa, Alberto Rábano, Michèle Cazes, Alix Cloarec, Luis M. Ruilope, and Rafael García-Robles

To estimate the effects of UP269-6, a nonpeptide angiotensin II receptor antagonist, and captopril, a converting enzyme inhibitor, on the progression of nephropathy, 77 uninephrectomized diabetic rats were maintained for 8 months with plasma glucose levels from 300 to 500 mg/dL. Systemic and renal parameters were periodically measured, and, at the time of death, a histological evaluation of renal damage was performed. Control rats (no additional treatment but insulin) showed increased blood pressure and urinary albumin levels, together with prominent alterations in the kidney (renal and glomerular hypertrophies, tubular atrophy, and 19% of sclerotic glomeruli). Captopril (50 mg/kg/day) and UP269-6 (10 mg/kg/day) reduced blood pressure and albumin excretion levels, and improved histological renal preservation (lower renal and glomerular hypertrophies, tubular atrophy, and percentage of sclerotic glomeruli: 5% and 7%, respectively).

Finally, a low dose of UP269-6 (1 mg/kg/day), which induced an intermediate level of blood pressure between control and the other treated groups, produced an equivalent degree of nephroprotection. Our data demonstrate the efficacy of this new angiotensin II receptor antagonist on the progression of diabetic renal damage. These results also reinforce the role attributed to angiotensin II in the development of renal derangement in this model, as UP269-6 is devoid of agonistic effect on the kinin system.

KEY WORDS: Captopril, angiotensin II receptor, UP269-6, Wistar rat, blood pressure, renal derangement, diabetes mellitus.

Insulin-dependent diabetes mellitus (IDDM) is a leading cause of the development of end-stage renal failure.1,2 The presence of arterial hypertension has been shown to be a concomitant risk factor for the progression of diabetic renal injury, as diverse antihypertensive therapies have demonstrated their ability to reduce the decline in renal function associated with established diabetic renal disease.3,4 However, several antihypertensive regimens have failed to prevent renal damage, even when a comparable level of blood pressure reduction was achieved.4–6 Among antihypertensive therapies, a higher efficacy of angiotensin converting enzyme inhibitors (CEIs) has been reported in the literature in experimental and human diabetic nephropathies.7–9

The possibility that this positive outcome is exclusively associated with blockade of angiotensin II (AII) generation is still controversial. In this regard, in addi-
tion to systemic effects that contribute to elevations in blood pressure, AII produces specific actions within the kidney that could enhance the severity of the lesions. So, together with its well-known efferent vasoconstriction, AII modulates mesangial cell contractility and macromolecular uptake, glomerular permeability to proteins, tubular sodium reabsorption, and vasa recta blood flow. Therapeutical regimens that modulate AII production, bioavailability, or activity could have renal protective effects through modification of these renal factors. The recent development of new antihypertensive drugs, such as the nonpeptide antagonists of the AII receptor, allows us to assess the contribution of the inhibition of AII bioactivity to the more positive outcome of CEIs in the retardation of renal damage progression. Preliminary studies have reported a renal preserving effect of these drugs in Sprague Dawley-diabetic rats, diabetic spontaneously hypertensive rats, as well as in models of renal mass ablation.

UP269-6 (UP) is a new orally active nonpeptide angiotensin II receptor (AT₁) antagonist, with no agonistic properties on AII and the kinin system. In the present study we have compared the effects of chronic treatment with this drug and with captopril (a CEI) on the progression of diabetic nephropathy in uninephrectomized streptozotocin-diabetic rats. Finally, we decided to investigate the effects of a low dose of UP in order to evaluate the repercussions of different levels of systemic blood pressure control on the severity of the renal damage.

METHODS

Animals The left kidneys of 77 age-matched Wistar rats with initial body weights (BW) ranging from 300 to 350 g were removed under pentobarbital anesthesia. This maneuver was performed to enhance and hasten the severity of diabetic glomerular lesions. Three weeks later, the animals were made diabetic by a single intravenous injection of 70 mg/kg BW streptozotocin (Sigma Chemical Co., St. Louis, MO) and 2 days later, successful induction of diabetes mellitus was confirmed by tail blood glucose (BG) measurement with a reflectometer meter (Miles Ames Division, Elkhart, IN). Throughout the experiment the rats were fed a standard pellet laboratory chow and allowed free access to water. Every animal received a daily injection of ultralente insulin, individually adjusted to maintain hyperglycemia (BG; 300 to 500 mg/dL), which was monitored twice a week. The first group (n = 17) received no treatment other than insulin (Control). A second group (n = 20) was additionally treated with a single dose of 50 mg/kg BW/day captopril (Captopril). A third group (n = 20) received a single dose of 10 mg/kg BW/day of UP269-6 (UPH). This dose was determined during preliminary studies to be a dose that matched the antihypertensive effect of captopril. Finally, a fourth group (n = 20) was given a single dose of 1 mg/kg BW/day of UP269-6 in order to obtain a lower decrease of blood pressure in the animals (UPL). Treatments other than insulin were administered by a gastric tube. The rats were kept under these conditions for 8 months, and then the animals were killed in order to assess the diabetic renal damage.

Analytical Periodical determinations of BW, BG, mean blood pressure (MBP) by tail-cuff method (Letica, Barcelona, Spain), and plasma creatinine by a reflectometer device (Reflotron, Boehringer Mannheim, Mannheim, Germany) were performed in every animal to estimate the evolution of systemic parameters. Every 8 weeks, all the animals were housed in metabolic cages, with free access to food and water, to collect 24-h urine for determining urinary creatinine and urinary albumin excretion (UₐbV). The latter was determined by a radioimmunodiffusion commercial kit specific for rat albumin (The Binding Site, Birmingham, England). At the time of death, the creatinine clearance (CrCl) was calculated from plasma and urine creatinine values, which were measured on the same day. In addition, glycosylated hemoglobin (HbA₁c) levels were determined.

Light Microscopy Remnant kidneys from all the rats were removed, weighed, and processed for light microscopy. Samples from every kidney were fixed by immersion in formaldehyde and embedded in paraffin. Sections were stained with hematoxylin-eosin, periodic acid-Schiff (PAS), and Masson trichrome. The presence of focal or diffuse glomerulosclerotic lesions was determined by scoring at least 75 superficial and midcortical glomeruli in two coronal sections. Glomerular volume estimation was performed by using an image analyzer (Microm, Barcelona, Spain), and values were obtained by measuring the same glomeruli used to assess histological damage. Maximum and minimum glomerular diameters between the internal edges of Bowman’s capsule were measured and the mean value of both was considered to be the diameter of each glomerulus. From this value, individual volumes were calculated for each glomerulus from the formula 4/3πr³. Tubular lesions were evaluated semiquantitatively by the degree of tubular atrophy as: none (−), slight (+), moderate (+ +), and intense (+ + +).

Statistical Analysis A Wilcoxon’s signed rank test for paired comparisons was used to analyze differences within the groups. Differences between the groups were tested by analysis of variance for multiple groups. P < .05 was considered significant. In the text, data are presented as mean ± SD.

RESULTS

Clinical and Biochemical Parameters (Table 1) The evolution of blood glucose levels and BW were parallel...
for the four groups during the 8 month follow-up without significant differences among them. The insulin supplementation required to maintain these comparable BG values was also fairly similar for the four groups. This equivalent metabolic situation was confirmed by the similar HbA1c levels shown by the four groups at the time of death. A significant increase in MBP was observed in the four groups (P < .05 v week), although the three treated groups showed significantly lower levels throughout the follow-up (P < .05 Control). The low dose of UP induced an intermediate level of blood pressure between the Control and the Capto and the UPH groups. The diabetic condition was associated with an elevation in 24-h urine and Na+ excretion in the four groups without differences among them during the study. Urinary albumin excretion (Figure 1) increased significantly (P < .05 v week 0) during the follow-up, although the three therapies evoked a comparable reduction in this parameter (P < .05 v Control). Finally, no difference in the creatinine clearance values were observed between the groups.

**Macroscopical and Histological Findings (Table 2, Figure 2)** The remaining kidneys obtained from the animals at the time of death showed a significant enlargement when compared with the contralateral one previously removed (P < .01) in all the groups. However, all the treatments exerted a diminution in the renal hypertrophy associated with the model (P < .05 v Control). This fact was confirmed by the lower glomerular hypertrophy observed in the three treated groups (P < .05 v Control). A moderate degree of tubular atrophy and metaplasia was present in the Control group, while these alterations were absent or exhibited a slight incidence in the treated groups. In addition, the percentage of sclerotic glomeruli (Figure 3) was significantly decreased in the treated groups (P < .01 v Control) without differences between them.

**DISCUSSION**

In the present study we have tested the effects of captopril and two different doses of UP269-6, an AII-receptor antagonist, on the progression of experimental diabetic nephropathy. We found that captopril exhibited nephroprotective actions that included the diminution of renal and glomerular hypertrophies, as well as a reduction in the renal damage (percentage of sclerotic glomeruli and tubulointerstitial lesions), when compared with kidneys from the Control group. In addition, ACEI treatment induced a decrease in mean arterial pressure and urinary albumin excretion. Angiotensin II receptor blockade, achieved with the high dose of UP, together with an equivalent reduction in blood pressure levels, produced a similar degree of preserving activity on the renal structure and function as those obtained with captopril. Finally, a comparable level of renal function and architectural preservation was achieved with a lower dose of UP, although this positive outcome was associated with an intermediate level of blood pressure between those of the Control and the UPH groups.

Renal injury is one of the most common features associated with experimental and human diabetes mellitus. Impaired renal functionality is usually related to hemodynamic and structural alterations within the kidney that occur during the progression of the disease. The key mechanism responsible for this derangement is still a matter of speculation, although

<table>
<thead>
<tr>
<th>Group</th>
<th>Week</th>
<th>Body Weight (g)</th>
<th>Mean Blood Pressure (mm Hg)</th>
<th>Blood Glucose (mg/dL)</th>
<th>HbA1c (%)</th>
<th>CrCl (mL/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>436.6 ± 52.4</td>
<td>115.0 ± 10.0</td>
<td>147.2 ± 52.4</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>516.0 ± 57.7</td>
<td>165.7 ± 25.4†</td>
<td>439.7 ± 155.1†</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>530.5 ± 71.2</td>
<td>182.3 ± 26.0†</td>
<td>491.2 ± 136.8†</td>
<td>8.7 ± 0.6</td>
<td>2.6 ± 0.47</td>
</tr>
<tr>
<td>CAPTO</td>
<td>0</td>
<td>444.9 ± 34.9</td>
<td>114.1 ± 11.1</td>
<td>141.7 ± 13.3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>514.3 ± 36.0</td>
<td>128.9 ± 31.6‡</td>
<td>435.9 ± 143.7†</td>
<td>—</td>
<td>—</td>
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<tr>
<td></td>
<td>32</td>
<td>509.2 ± 42.5</td>
<td>144.7 ± 20.6‡</td>
<td>596.8 ± 134.4†</td>
<td>8.5 ± 0.4</td>
<td>2.9 ± 0.63</td>
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<td>UPH</td>
<td>0</td>
<td>431.6 ± 36.3</td>
<td>118.2 ± 12.3</td>
<td>151.6 ± 21.8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>506.8 ± 40.6</td>
<td>124.6 ± 28.4‡</td>
<td>414.8 ± 163.5†</td>
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<tr>
<td></td>
<td>32</td>
<td>503.0 ± 41.8</td>
<td>137.6 ± 15.8‡</td>
<td>527.2 ± 64.5†</td>
<td>8.4 ± 0.7</td>
<td>2.4 ± 0.73</td>
</tr>
<tr>
<td>UPL</td>
<td>0</td>
<td>432.5 ± 38.4</td>
<td>117.3 ± 14.5</td>
<td>139.17 ± 11.9</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>517.4 ± 56.0</td>
<td>141.5 ± 18.6†</td>
<td>424.2 ± 127.9†</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>536.0 ± 97.2</td>
<td>159.6 ± 20.1‡</td>
<td>544.4 ± 129.4†</td>
<td>8.9 ± 0.5</td>
<td>2.6 ± 0.46</td>
</tr>
</tbody>
</table>

HbA1c, Glycosylated hemoglobin; CrCl, creatinine clearance; CAPTO, captopril group; UPH, 10 mg/kg body weight/day UP269-6 group; UPL, 1 mg/kg body weight/day UP269-6 group.

* P < .05 v 0 week.
† P < .001 v 0 week.
‡ P < .05 v control.
sustained hyperglycemia and hypertension are recognized risk factors that accelerate the progression rate of diabetic renal disease. However, not all antihypertensive therapies have shown similar efficacies in retarding this progression, despite the comparable reductions of blood pressure levels. In this respect, CEIs have demonstrated their ability to slow the decline in renal functionality associated with this pathology with a diminution of urinary albumin excretion and amelioration of size-selective properties of the glomerular barrier. Nevertheless, this group of drugs exerts actions other than the direct blockade of the pressor effects of AII. Some extent of the positive outcome obtained with CEIs, such as reduction in protein excretion, has been attributed to the potentiation of the bradykinin system exerted by these drugs. The relative role played by these or even other mechanisms in the prevention of renal diabetic injury is still controversial.

Nonpeptide AII receptor (AT₁) antagonists, in addition to their therapeutical application, can be useful tools for estimating the specific contribution of AII to the progression of renal functionality loss. In our model, UP induced nephroprotective actions comparable to those achieved with captopril in blood pressure and glucose-matched conditions. Our data are in agreement with previously published results which reported nephroprotective actions of AII receptor antagonist therapies in diabetic rats. The low dose of UP269-6 attenuated the progression of diabetic nephropathy in a similar manner to the UPH and Capto groups, as reflected by comparable levels of albumin excretion, renal and glomerular hypertrophies, and the incidence of renal lesions. This positive outcome was not associated with equivalent reductions in blood pressure levels. The present result indicates that, although important, blood pressure control is not the only mechanism responsible for diabetic renal injury. In agreement with this statement, it has been previously reported that therapies not affecting systemic blood pressure levels induced nephroprotective actions in diabetic models. Indeed, thromboxanes

**TABLE 2. MACRO- AND MICROSCOPIC RENAL PARAMETERS**

<table>
<thead>
<tr>
<th>Group</th>
<th>Kidney Weight (g)</th>
<th>Kidney Weight/Body Weight Index (g/kg)</th>
<th>Glomerular Volume ($\mu m^3 \times 10^6$)</th>
<th>Tubular Lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.0 ± 0.7</td>
<td>7.5 ± 0.3</td>
<td>4.9 ± 1.2</td>
<td>++</td>
</tr>
<tr>
<td>CAPTO</td>
<td>3.4 ± 0.6*</td>
<td>6.6 ± 0.1*</td>
<td>3.7 ± 0.4*</td>
<td>−/+</td>
</tr>
<tr>
<td>UPH</td>
<td>3.3 ± 0.5*</td>
<td>6.5 ± 0.2*</td>
<td>3.9 ± 0.6*</td>
<td>−/+</td>
</tr>
<tr>
<td>UPL</td>
<td>3.3 ± 0.6*</td>
<td>6.4 ± 0.3*</td>
<td>3.7 ± 0.5*</td>
<td>+</td>
</tr>
</tbody>
</table>

See Table 1 for abbreviations. (−) No; (+) Slight; (+++) Moderate.

* P < .05 vs control.
have been implicated in the progression of renal injury, as their inhibitors are able to decrease urinary albumin excretion.\(^{25,26}\) Furthermore, our group has documented that chronic treatment with cicaprost, a PG\(_I\_2\) analog, not only reduced urinary albumin, but also reduced the incidence of glomerular sclerosis and renal and glomerular hypertrophies in the absence of blood pressure control.\(^{27,28}\) Finally, reductions in blood pressure levels with losartan did not evoke glomerular protection in a different model of type II diabetes,\(^{29}\) reinforcing the statement that in some settings the extent of diabetic renal injury can be dissociated from the level of blood pressure.

The renal preserving effect induced by both doses of UP seems to support the fundamental and direct implication of AII in the pathogenesis of kidney derangement, as this group of drugs does not potentiate the bradykinin system as do the CEIs. Angiotensin II contributes to the elevation of intraglomerular pressure, which is considered a fundamental factor in the pathogenesis of diabetic renal damage by its preferential vasoconstrictor effect in the efferent arterioles. This early alteration can be enhanced and perpetuated by the trophic effects of AII in the glomerular mesangium and in the renal vasculature.\(^{10}\) In this regard, UP269-6 may reduce this intraglomerular pressure, as do other AII receptor antagonists,\(^{30}\) thus preventing glomerular size-selective functionality,\(^{14}\) which will further contribute to nephroprotection. The present rat model has been reported to involve an overexpression of the renal tissue renin-angiotensin system, with specific glomerular and vascular locations.\(^{50}\) This evidence potentially explains the beneficial response to both captopril and UP achieved in our study, as both drugs induce a blockade of AII generation or action.

All these data indicate that the progression of diabetic nephropathy is a multifactorial process in which...
FIGURE 3. Mean percentage values of sclerotic glomeruli in diabetic rats (Control), and diabetic rats treated with captopril (Capto), or high (UPH) and low (UPL) doses of UP269-6.

several mechanisms are involved. For this reason, diverse therapies that could act on distinct systems will exert different degrees of renal protection according to the relative contribution of each of these systems to the diabetic renal derangement.

In summary, we have demonstrated in a model of experimentally-induced diabetes mellitus that UP269-6, a new orally active nonpeptide AII receptor antagonist, induced a comparable degree of renal preservation to that afforded by captopril, as estimated by functional and histological parameters. This nephroprotection was achieved with two different doses of UP, which maintained blood pressure at two levels, similar to or higher than that achieved with captopril. Our data reinforces the role that AII plays in the pathogenesis of diabetic renal damage.

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REFERENCES


