Additive Antiproteinuric Effect of Pentoxifylline in Patients with Type 2 Diabetes under Angiotensin II Receptor Blockade: A Short-Term, Randomized, Controlled Trial

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Despite the beneficial effects of blockade of the renin-angiotensin system in diabetic nephropathy (DN), albuminuria and progression of renal disease are not completely halted by these agents. Therefore, it is necessary to explore potential antiproteinuric and renoprotective effects of innovative therapeutic approaches. This study tested the hypothesis that the combination of pentoxifylline (PTF) with angiotensin II receptor blockers in normotensive patients with type 2 diabetes produces an additive antiproteinuric effect. Sixty-one patients with DN and residual albuminuria despite treatment with the recommended doses of ARB for >1 yr were randomly assigned to receive the addition of 1200 mg of PTF daily (n = 30) or to a control group (n = 31). Baseline characteristics were similar between groups, and correlation analysis showed a significant association between urinary albumin excretion (UAE) and urinary TNF-α (R = 0.53, P < 0.001). After 4 mo, albuminuria showed a significant decrease in patients who received PTF, from 900 mg/24 h (466 to 1542 mg/d) to 791 mg/24 h (309 to 1400 mg/d; P < 0.001), whereas no significant changes were observed in the control group: 920 mg/24 h (450 to 1489 mg/d) at baseline, and 900 mg/24 h (428 to 1800 mg/d) at the end of the study. The mean percentage variation of UAE in the treatment and control groups was −16.7 and 5.5%, respectively (between-group comparison, P < 0.001). This additive antiproteinuric effect was not dependent on changes in BP or metabolic control. However, both serum and urinary levels of TNF-α also decreased in patients who received PTF, from 6.4 pg/ml (2.1 to 9.7) and 16 pg/mg (8 to 29) at baseline to 4.6 pg/ml (0.4 to 9) and 14.2 pg/mg (3 to 26) at the end of the study, respectively (P < 0.01), without significant variations in control patients. Moreover, regression analysis of the study showed a correlation between the change in UAE and the change in urinary TNF-α in patients who were treated with PTF (R = 0.49, P < 0.001). In conclusion, administration of PTF to patients who have type 2 diabetes and are under long-term treatment with an ARB produces a significant additive antiproteinuric effect associated with a reduction of urinary TNF-α excretion.


Nephropathy associated with type 2 diabetes is the most frequent cause of ESRD, and extrapolations suggest that this number will multiply in the future. In the past few decades, our knowledge of diabetic nephropathy (DN) and its clinical course, the factors that influence it, and the possibilities of treatment have improved substantially. From a pathophysiologic point of view, the importance of metabolic and hemodynamic factors for the risk for developing DN have become clear, with urinary protein excretion being one of the most important predictors for progressive renal disease (1).

There now are convincing data that type 2 diabetes includes an inflammatory component (2–4). Moreover, this inflammatory condition has been related to diabetic complications, including nephropathy. Several studies have reported that serum levels of inflammatory markers are higher in patients with diabetes and increased urinary albumin excretion (UAE) when compared with normoalbuminuric individuals (5–7). Furthermore, findings from the Insulin Resistance Atherosclerosis Study showed an association of C-reactive protein and fibrinogen with UAE in individuals with type 2 diabetes and microalbuminuria (6). Finally, in a previous study, we found that urinary TNF-α level was an independent predictor of UAE in individuals with type 2 diabetes (7). Therefore, inflammation emerges as a potential mechanism in the pathogenesis of renal injury in type 2 diabetes (7,8).

Albuminuria is an independent risk factor for the progression of renal disease in patients with diabetes and nephropathy (9). Interventions that have ameliorated the progression of DN have always been associated with a reduction in urinary protein excretion (10), with residual albuminuria during treatment being a predictor of the rate of decline in the GFR (9). Therefore, renoprotective therapy should aim to achieve the maximal antiproteinuric effect (11). Recent prospective randomized studies have shown that the interruption of the renin-angiotensin system (RAS) with angiotensin II receptor blockers (ARB) in patients with type 2 diabetes and overt nephropathy delays the
progression of renal disease (12,13). However, blockers of the RAS provide an imperfect protection; therefore, innovative approaches to prevention are necessary (14).

Pentoxifylline (PTF) is a methylxanthine derivative with favorable effects on microcirculatory blood flow as a result of its rheological properties (15). Recent studies have shown that PTF reduces urinary protein excretion in individuals with diabetes, both with normal renal function (16–18) and with renal insufficiency (19). In a previous study in patients with diabetes and nephropathy, we found that TNF-α concentrations were significantly elevated and related to the urinary protein excretion and, moreover, that the levels of this cytokine decreased after PTF administration, with a significant correlation between the decrease of TNF-α and the reduction of proteinuria (19).

This study was undertaken to evaluate whether in normotensive patients with diabetes and residual albuminuria despite adequate therapy with ARB, the addition of PTF provides an additive antiproteinuric effect. The hypothesis is that this combined therapy may offer a greater antiproteinuric action, which is related to modulation of inflammatory parameters, specifically TNF-α.

Materials and Methods

Patients
A previous sample size calculation to detect a 25% relative difference in the change in UAE rate with an α value of 0.05 and a β value of 0.80 showed a need for a minimum of 26 patients. The criteria for the selection of patients were diabetic nephropathy, defined by persistent albuminuria >300 mg/24 h in two consecutive determinations, no other kidney or renal tract disease, and presence of diabetic retinopathy (20,21) and normal BP (≥140/90 mmHg); treatment with the recommended doses of ARB for >1 yr; normal renal function, defined as a GFR ≥90 ml/min (calculated using the Modification of Diet in Renal Disease study equation) (22); and insufficient response to conventional therapy, defined as albuminuria >400 mg/24 h in three consecutive measurements in the 3 mo before inclusion in the study.

Exclusion criteria were current acute illness (including infectious diseases), medical history of cardiovascular disease (cardiac, cerebral, or peripheral vascular disease), and history of cigarette smoking. Before the definitive inclusion, the possible existence of immunologic diseases, malignancy, and infections was investigated. White blood cell count was <10,000/mm³ in all cases. Determination of tumor markers including carcino-embryonic antigen, α-fetoprotein, cancer antigen 125, and prostate-specific antigen were negative. Serologic tests for antinuclear antibodies, antineutrophil cytoplasmic antibodies, cryoglobulins, rheumatoid factor, Ig, and complement were negative or within the normal range. Urine cultures and serology to hepatitis B, hepatitis C, and HIV were also negative.

Study Design
The study was prospective and randomized, and it was performed in a single center. The protocol was in accordance with the Declaration of Helsinki and the local committee, and all patients gave informed consent before participating in the study. After an initial 2-wk run-in period, patients were randomized using a computer-generated random-number table into a control group or an active group. Patients in the control group (n = 30) were maintained at their scheduled ARB dosage with no additional treatment, whereas patients in the active group (n = 31) were invited to add 600 mg of PTF twice daily for 4 mo.

All patients were insulin dependent and received at least two daily injections of human insulin. There were no changes in the treatment during the study.

Body mass index (BMI) was calculated as weight (kg)/height (m²). Arterial BP was measured by a mercury sphygmomanometer by the same observer with the patient in the sitting position after 5 min of rest. Three readings separated by 2 min were taken, and the average was used for calculation. The first appearance of sound (phase 1) was used to define systolic BP. The disappearance of sound (phase 5) was used to define diastolic BP.

Blood samples were drawn from each patient before breakfast in the morning (between 8 and 11 a.m.), after an 8- to 12-h overnight fast. Samples were collected in sterile tubes, centrifuged at 3000 × g for 10 min at 4°C, and then stored at −70°C until assayed. The plasma glucose level was measured by an automated enzymatic method. The glycated hemoglobin (HbA1c) concentration was measured by HPLC. The serum TNF-α concentration was determined with a high-sensitivity quantitative sandwich enzyme immunoassay (R&D Systems, Minneapolis, MN). The lower limit of detection is 0.4 pg/ml (0.02 pmol/L), and the intra- and interassay coefficients of variation of the assay were 5.6 and 8.1%, respectively.

UAE was determined by two consecutive 24-h urine collections at the end of each period, and the mean value was computed. After collection, the samples were centrifuged at 3000 × g for 10 min and the supernatant was stored at −20°C. Urinary albumin was quantified by immuno-turbidimetry (coefficient of variation 5.5%). ELISA was used for the detection of urinary TNF-α, which was related to the concomitant urinary creatinine content to compensate for alterations caused by varying urinary concentration and therefore is expressed as pg/mg.

Statistical Analyses
Results are presented as mean ± SD, except for albuminuria and TNF-α, which are presented as median and range. Differences in parameters between groups were analyzed by the t test and the Mann-Whitney U test when appropriate. Changes in parameters within groups from the basal to the end of the study were analyzed by paired t test or Wilcoxon test, as appropriate. Correlation between variables was calculated using the Pearson and the Spearman correlation tests. P < 0.05 was considered statistically significant. Statistical analysis were conducted using Statistica 5.5 (Statsoft Inc., Tulsa, OK).

Results
A total of 102 patients were initially evaluated. Forty-one were excluded because of a medical history of cardiovascular disease, cigarette smoking, elevated prostate-specific antigen, and positive serology for hepatitis C. Sixty-one patients were finally randomized in the trial; all of them completed the study and were included in the statistical analysis (Figure 1). All of the patients received treatment with ARB at the recommended dosage (irbesartan 300 mg, 31 patients; losartan 100 mg, 26 patients; and candesartan 16 mg, 18 patients). The baseline demographic, clinical, and laboratory characteristics of the two groups were similar (Table 1). There were no significant differences in BP, renal function, or metabolic control. All patients showed residual albuminuria despite treatment with ARB at the maximal dosage for >1 yr. UAE was similar in both groups: 900 mg/d (range 466 to 1542 mg/d) in the treatment group and 910 mg/d (range 450 to 1489 mg/d) in the control group. Likewise, the serum and urinary levels of TNF-α were similar between groups.
UAE showed a significant decrease in the treatment group: 900 mg/d (466 to 1542 mg/d) at baseline to 791 mg/d (309 to 1400 mg/d) at the end of the study ($P < 0.001$). No significant changes in UAE were observed in the control group: 910 mg/d (450 to 1489 mg/d) at baseline and 900 mg/d (428 to 1800 mg/d) at the end of the study (Figure 2). The percentage variations of UAE in the treatment and control groups were $-16.7$ and $5.5\%$, respectively (between-groups comparison, $P < 0.001$). Both serum and urinary levels of TNF-$\alpha$ also decreased in patients who received PTF, from 6.4 pg/ml (2.1 to 9.7) and 16 pg/mg (8 to 29) at baseline to 4.6 pg/ml (0.4 to 9) and 14.2 pg/mg (3 to 26) at the end of the study, respectively ($P < 0.01$). On the contrary, these parameters did not show significant variations in control patients (Table 2). The mean percentage variation of serum and urinary TNF-$\alpha$ were $-14.9$ and $-13\%$ in PTF-treated patients, respectively. There were no changes in any group during the study regarding BP, metabolic control, or renal function.

We compared the variation of BP and HbA$_{1c}$ between patients who received PTF and control subjects. After analysis, no significant differences were found between the groups, and no relationship was observed between the level of BP at baseline and the changes in albuminuria. In addition, we compared these variations in patients who showed a mean percentage reduction of UAE $>15\%$ after PTF administration with respect to patients with a decrease of albuminuria $<15\%$. Similarly, there were no differences between the groups, and no significant association was found between these parameters.

After PTF administration, UAE rose in five patients, with a mean increase of $4.7\%$, similar to the mean variation observed in patients who did not receive PTF ($5.5\%)$. On the contrary, 25 patients showed a reduction of UAE. The mean percentage reduction was $>15\%$ in 15 patients and $>30\%$ in nine patients. Clinical characteristics of patients who showed the best response to PTF (mean UAE reduction $>15\%$) were similar to those in the other 15 patients, with no differences in baseline

### Table 1. Clinical characteristics of patients at baseline$^a$

<table>
<thead>
<tr>
<th></th>
<th>PTF-Treated Group ($n = 30$)</th>
<th>Control Group ($n = 31$)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>58.6 ± 9.0</td>
<td>58.8 ± 6.0</td>
<td>NS</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>16/14</td>
<td>15/16</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes time (yr)</td>
<td>13.1 ± 3.3</td>
<td>12.5 ± 3.4</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>134.4 ± 6.0</td>
<td>132.1 ± 6.2</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>83.3 ± 6.7</td>
<td>81.5 ± 7.6</td>
<td>NS</td>
</tr>
<tr>
<td>SCr (mg/dl)</td>
<td>0.98 ± 0.20</td>
<td>1.0 ± 0.17</td>
<td>NS</td>
</tr>
<tr>
<td>HbA$_{1c}$ (%)</td>
<td>8.02 ± 0.97</td>
<td>8.07 ± 1.01</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>30.4 ± 2.1</td>
<td>29.3 ± 1.8</td>
<td>NS</td>
</tr>
<tr>
<td>UAE (mg/24 h)</td>
<td>900 (466 to 1542)</td>
<td>920 (450 to 1489)</td>
<td>NS</td>
</tr>
<tr>
<td>UTNF-$\alpha$ (pg/mg)</td>
<td>15.0 (7.0 to 29.0)</td>
<td>14.0 (5.0 to 32.0)</td>
<td>NS</td>
</tr>
<tr>
<td>STNF-$\alpha$ (pg/ml)</td>
<td>6.4 (2.1 to 9.7)</td>
<td>5.1 (1.4 to 10.0)</td>
<td>NS</td>
</tr>
</tbody>
</table>

$^a$PTF, pentoxifylline; Scr, serum creatinine; HbA$_{1c}$, glycosylated hemoglobin; BMI, body mass index; UAE, urinary albumin excretion; UTNF-$\alpha$, urinary TNF-$\alpha$; STNF-$\alpha$, serum TNF-$\alpha$. 
characteristics regarding age, gender, duration of diabetes, BP, serum creatinine, metabolic control, BMI, UAE, or TNF-α levels.

Baseline UAE was significantly associated with duration of diabetes (R = 0.37, \(P < 0.01\)), HbA1c (R = 0.45, \(P < 0.001\)), and urinary TNF-α (R = 0.53, \(P < 0.001\)). It is interesting that there was no association between serum and urinary levels of TNF-α. Finally, linear regression analysis at the end of the study showed a correlation between the change in UAE and the change in urinary TNF-α in patients who were treated with PTF (R = 0.49, \(P < 0.001\)).

PTF was well tolerated in the patients who were assigned to the treatment group. No serious side effects were observed. Four patients developed dizziness, and three patients complained of dyspepsia. These symptoms were transient in all cases, and no patient withdrew from the study as a result of PTF side effects.

**Discussion**

Interventions that prevent or palliate DN will have a positive impact by improving patient care and diminishing health care costs. The reduction of urinary protein excretion when evaluating the renoprotective properties of treatment is extremely important. Amelioration of the progression of renal disease has always been associated with a decrease of proteinuria (10,23). Furthermore, in diabetic renal disease, residual albuminuria during treatment is a predictor of the decline in GFR (9); therefore, UAE should be reduced as far as possible (11). Recent studies have demonstrated clearly the antiproteinuric effect and the renoprotective efficacy of ARB in patients with type 2 diabetes (12,13). However, a residual proteinuria was still detectable at the end of these studies, and despite the accumulating evidence of their efficacy, blockers of the RAS are considered to provide imperfect protection (14). Our study shows in a prospective and randomized design an additional effect on the reduction of UAE of treatment with PTF in a group of patients with type 2 diabetes and DN and residual albuminuria despite long-term therapy with ARB at the recommended dosage. We must stress the preliminary nature of these findings, especially when only one previous study has analyzed the effect of combining PTF with a blocker of the RAS (24). In that study, 50 patients with type 2 diabetes and persistent microalbuminuria were randomly assigned to either the angiotensin-converting enzyme inhibitor lisinopril (10 mg/d) or a PTF (600 mg/d) and lisinopril (10 mg/d) combined group. After 9 mo, the mean percentage reduction of proteinuria in the lisinopril group was 35%, whereas in the lisinopril + PTF group, it was 41% (\(P < 0.05\)). This additional 6% decrease of UAE in patients who received PTF was not associated with changes in hemodynamic or metabolic parameters. In our study, the patients were normotensive at study entry, and during the follow-up, no changes were observed in BP or HbA1c. Furthermore, there was no significant relationship between the variation of UAE with BP at baseline or with changes in BP during the study. Therefore, the additive antiproteinuric effect of PTF was not dependent on a reduction of BP or an improvement in metabolic control. Previous studies have shown that PTF administration is not associated with changes of these parameters in patients with different conditions: Normal individuals, septic patients, patients with diabetes and normal renal function, proteinuria or renal failure, patients with idiopathic dilated cardiomyopathy, and cardiac transplant patients (17,19,25–28).

There has been increasing interest in the past few years in the relevance of inflammation in diabetes (2) and its role in the pathogenesis of diabetic complications, including nephropathy (7,8). Increased concentrations of inflammatory parameters (C-reactive protein, sialic acid, fibrinogen, TNF-α, IL-1, and IL-6) have been reported in patients with diabetes (3–6,29). Recent studies have shown that levels of these substances increase as nephropathy progresses (6,30,31), with an independent rela-

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**Table 2. Evolution of study parameters in the active and control groups**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Fourth Month</th>
<th>(P)</th>
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</thead>
<tbody>
<tr>
<td><strong>PTF group (n = 30)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>systolic BP (mmHg)</td>
<td>134 ± 6</td>
<td>135 ± 5</td>
<td>NS</td>
</tr>
<tr>
<td>diastolic BP (mmHg)</td>
<td>83 ± 6</td>
<td>84 ± 5</td>
<td>NS</td>
</tr>
<tr>
<td>Scr (mg/dl)</td>
<td>0.98 ± 0.20</td>
<td>1.0 ± 0.18</td>
<td>NS</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>8.0 ± 0.9</td>
<td>7.9 ± 0.9</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.4 ± 2.1</td>
<td>30.0 ± 2.4</td>
<td>NS</td>
</tr>
<tr>
<td>UTNF-α (pg/mg)</td>
<td>16.0 (8.0 to 29.0)</td>
<td>14.2 (3.0 to 26.0)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>STNF-α (pg/ml)</td>
<td>6.4 (2.1 to 9.7)</td>
<td>4.6 (0.4 to 9.0)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td><strong>Control group (n = 31)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>systolic BP (mmHg)</td>
<td>132 ± 6</td>
<td>133 ± 4</td>
<td>NS</td>
</tr>
<tr>
<td>diastolic BP (mmHg)</td>
<td>81 ± 7</td>
<td>82 ± 6</td>
<td>NS</td>
</tr>
<tr>
<td>Scr (mg/dl)</td>
<td>1.0 ± 0.17</td>
<td>1.03 ± 0.14</td>
<td>NS</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>8.0 ± 1.0</td>
<td>8.0 ± 0.9</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.3 ± 1.8</td>
<td>29.3 ± 1.7</td>
<td>NS</td>
</tr>
<tr>
<td>UTNF-α (pg/mg)</td>
<td>15.0 (7.0 to 29.0)</td>
<td>14.0 (5.0 to 32.0)</td>
<td>NS</td>
</tr>
<tr>
<td>STNF-α (pg/ml)</td>
<td>5.1 (1.4 to 10.0)</td>
<td>5.5 (2.5 to 9.9)</td>
<td>NS</td>
</tr>
</tbody>
</table>
tionship between inflammatory parameters and UAE (6,7). Evidence now indicates that inflammatory phenomena are important to translate the primary metabolic and hemodynamic insults into renal damage in DN (32–36).

In 1991, Hasegawa et al. (37) reported that peritoneal macrophages that were incubated with glomerular basement membranes from diabetic rats produced significantly greater levels of TNF-α and IL-1 than did macrophages that were incubated with membranes from normal rats. These authors suggested for the first time that proinflammatory cytokines could participate in the development of DN. Recent studies have highlighted the potential relevance of TNF-α in the development and progression of renal lesion in diabetes. Endothelial, mesangial, glomerular, and tubular epithelial cells are able to produce cytokines, and diverse experimental works have demonstrated that renal mRNA levels for TNF-α are significantly increased in diabetic rats compared with control rats (37–40). From a pathogenic perspective, it is particularly relevant that the findings of experimental studies show that increased urinary as well as renal interstitial concentrations of TNF-α precede the rise in albuminuria (32,33). Finally, it is important to take into account that TNF-α is cytotoxic to glomerular, mesangial, and epithelial cells and may induce significant renal damage (41,42). Moreover, McCarthy et al. (43) demonstrated a direct harmful effect of TNF-α on the protein permeability barrier of the glomerulus, which is independent from alterations in hemodynamic factors or effects of recruited inflammatory cells.

Experimental and clinical studies have demonstrated that DN exhibits signs of inflammation, such as increased expression of adhesion molecules (44), chemokines (45), growth factors (46), and proinflammatory cytokines, including TNF-α, in renal tissue (37–40). In addition, the enhanced passage of proteins across the glomerular barrier as a result of any insult to the filtration compartment exerts deleterious effects on the kidney in part by eliciting an inflammatory reaction with an increased gene expression for several proinflammatory molecules (47,48). In a previous study, Remuzzi et al. (49) demonstrated in a proteinuric nonimmune model of progressive renal disease that the inhibition of RAS by lisinopril limited proteinuria, interstitial inflammation, and severe lesions, whereas the disease was completely suppressed only when lisinopril was combined with mycophenolate mofetil (MMF). Moreover, in a recent study, Utimura et al. (50) evaluated the anti-inflammatory and hemodynamic effects of MMF administration to streptozotocin-induced diabetic rats. The results showed that MMF prevented the development of albuminuria and glomerular injury in diabetic rats, which were reduced to levels that were indistinguishable from those seen in age-matched nondiabetic controls. It is worth noting that these beneficial effects could not be ascribed to hemodynamic or metabolic factors, indicating that they resulted directly from the immunosuppressive/anti-inflammatory properties of MMF.

In this study, UAE showed a significant mean percentage decrease of 16.7% in the PTF-treated group, whereas there was a nonsignificant 5.5% increase in the control group (P < 0.001 between groups). Our results confirm previous findings reporting an antiproteinuric effect of PTF in patients with DN (16–18). This antiproteinuric effect has been related to an increase in erythrocyte deformability with reduction of blood viscosity and a subsequent decrease of glomerular hydraulic pressure (51), to a blockade of adenosine receptors (52), and based on its anti-TNF-α properties (53,54), the modulation of immune and inflammatory responses has also been suggested as a potential antiproteinuric mechanism of PTF (7,8,19). In our study, in addition to a decrease of UAE, a significant reduction of serum and urinary TNF-α levels was observed in patients who received PTF (14.9 and 13%, respectively). On the contrary, these parameters did not show significant changes in control subjects (P < 0.05 between groups). Furthermore, in agreement with previous data (18,19), there was a positive and significant correlation between the change in UAE and the change in urinary TNF-α in patients who were treated with PTF (R = 0.49, P < 0.001). It is important to note that there was no significant correlation between the serum and urinary concentrations of TNF-α, which suggests an intrarenal production of this proinflammatory cytokine.

PTF is a methylxanthine phosphodiesterase inhibitor that is used clinically to treat patients with peripheral vascular disease (15). In addition to its hemorheologic activity, PTF possesses anti-inflammatory and immunoregulatory properties. In vivo studies have shown beneficial effects of PTF in different models of renal disease, including murine lupus nephritis (55), crescentic glomerulonephritis (56), and mesangial proliferative glomerulonephritis (57). A recent work in rats with a remnant kidney demonstrated the significant effects of PTF in modulating inflammation, cell proliferation, and fibrosis (58). In particular, PTF administration attenuated interstitial inflammation, downregulated monocyte chemoattractant protein-1 gene expression, reduced the expression of mitogenic and profibrogenic genes, and suppressed the proliferation of interstitial fibroblast and glomerular mesangial cells. Moreover, therapy combining PTF with the angiotensin-converting enzyme inhibitor cilazapril dramatically attenuated proteinuria and almost completely prevented renal disease progression. However, parallel to these experimental studies, clinical trials in patients with DN have also shown that PTF attenuates proteinuria (16–18) and reduces endogenous TNF-α (19).

This study is limited by the lack of information regarding the antiproteinuric effect of ARB before the addition of PTF. Despite this limitation, several considerations can be made on the basis of the results of previous studies. Recent trials have analyzed the renoprotective efficacy of losartan (13), irbesartan (12), and candesartan (59). These studies showed that the mean reduction of UAE was 34, 33, and 59%, respectively. Based on these results, the additional 16.7% reduction after PTF therapy observed in our study may be considered significant, because it would represent a 49.1, 50.6, and 28.3% of the reduction of UAE previously achieved with the use of losartan, irbesartan, and candesartan, respectively. In addition, any further reduction of albuminuria in patients with diabetes is of great importance. The recent study by de Zeeuw et al. (60) demonstrated that UAE is the most powerful marker for subsequent renal events in patient with type 2 diabetes and nephropathy and that the degree of albuminuria reduction is linearly related to the sub-
sequent renal protection. Moreover, the degree of residual albuminuria after ARB therapy remained proportionally associated with renal risk. Therefore, reduction of the residual albuminuria by additional approaches needs to be considered, because it would be associated with additional end-organ protection. Conversely, recent studies have found that dual blockade of the RAS resulted in a greater reduction of UAE (between 14 and 28%) compared with that observed with monotherapy (59,61,62). In our study, the additional decrease of albuminuria after addition of PTF is similar to those reported with dual blockade of the RAS.

Finally, a question of interest is whether the additive antiproteinuric effect of PTF is sustained over time. Unfortunately, data on this aspect are scarce. Two previous prospective studies showed that PTF administration to patients with diabetes and nephropathy was associated with a significant reduction of proteinuria after 6 mo of follow-up (19,26). More interesting, Gorson (63) reported in two patients who were treated with PTF over a long period (12 and 18 mo, respectively) that urinary protein excretion exhibited a marked and progressive reduction, with a percentage decrease >70% with respect to the baseline in both cases.

In conclusion, the results of our short-term study, although of a preliminary nature, show that in patients who have type 2 diabetes and are under long-term treatment with an ARB, the administration of PTF has a significant additive antiproteinuric effect not related to the decrease of BP or an improvement of metabolic control. This beneficial effect was associated with a reduction of urinary TNF-α excretion. On the basis of our results, as well as of those of previous studies, it is interesting to speculate that PTF, via its ability to antagonize TNF-α and to modulate other inflammatory and immune mediators, may have therapeutic implications for DN. Further clinical investigation is necessary to determine whether this innovative approach might be associated with significant renoprotective effects.

Acknowledgments
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Utimura R, Fujihara CK, Mattar AL, Malheiros DM, No-


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