Effect of losartan on TGF-β1 and urinary albumin excretion in patients with type 2 diabetes mellitus and microalbuminuria

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Abstract

Background. The aim of the present study was to determine the effect of losartan on transforming growth factor-β1 (TGF-β1) plasma levels and urinary albumin excretion (UAE) in patients with type 2 diabetes mellitus, mild hypertension and microalbuminuria.

Methods. Fourteen patients (eight males, aged 55 ± 6 years) with type 2 diabetes mellitus, mild arterial hypertension and microalbuminuria, participating in an open, uncontrolled, pilot study were included. Patients were treated for 8 weeks with losartan. TGF-β1 plasma levels, UAE and 24-h blood pressure monitoring were determined at baseline and at 4 and 8 weeks.

Results. At 4 and 8 weeks of treatment, a reduction was observed in TGF-β1 plasma levels (5.5 ± 4.5 vs 2.0 ± 0.6 and 2.6 ± 1.0 ng/ml, P < 0.005), UAE (96 ± 65 vs 59 ± 59 and 64 ± 47 μg/min, P < 0.01), 24-h systolic blood pressure (136 ± 9 vs 129 ± 9 and 130 ± 10 mmHg, P < 0.01) and 24-h diastolic blood pressure (77 ± 9 vs 74 ± 8 and 74 ± 7 mmHg, P < 0.03). Stratifying the patients by baseline TGF-β1, seven had TGF-β1 plasma values higher than normal controls. At 4 and 8 weeks, they showed a marked reduction in TGF-β1 values (9.0 ± 3.9 to 2.1 ± 0.7 and 2.5 ± 0.7 ng/ml, P < 0.01) and UAE (106 ± 83 to 49 ± 42 and 38 ± 26 μg/min, P < 0.05), with good correlation between the percentage reduction of both parameters (r = 0.83, P < 0.01). The remaining seven patients, with normal baseline TGF-β1 plasma levels, showed no change in TGF-β1 plasma levels and UAE after treatment.

Conclusion. Treatment with losartan decreases TGF-β1 plasma values and UAE in type 2 diabetes mellitus patients with high baseline TGF-β1 levels, suggesting that TGF-β1 may be a marker to detect patients who may particularly benefit from renin-angiotensin system blockade.

Keywords: hypertension; losartan; microalbuminuria; transforming growth factor-β1; type 2 diabetes

Introduction

Transforming growth factor-β1 (TGF-β1) is the main fibrogenetic cytokine which is overproduced in diabetes mellitus and is one of the factors that has been implicated in the pathogenesis of diabetic nephropathy (DN) [1]. It has been demonstrated in both animal models and humans that TGF-β1 induces renal cell hypertrophy, stimulates the synthesis of extracellular matrix components and blocks matrix degradation by stimulating protease inhibitors such as the plasminogen activator inhibitor-1 (PAI-1) [2] and the tissue inhibitor of metalloproteinase-1 (TIMP-1) [3]. Therefore, drugs which are able to inhibit or neutralize TGF-β1 activity may be useful in preventing the appearance and progression of DN. In this regard, treatment with anti-TGF-β1 antibodies in animal models prevents the effects of high glucose, inducing cellular hypertrophy and stimulating collagen biosynthesis. Moreover, the glomerular proteoglycan, decorin, binds TGF-β1 and neutralizes its activity in rats with glomerulonephritis induced by the anti-Thy 1.1 antibody [4–6].

Experimental animal and in vitro studies recently have demonstrated that angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor antagonists may decrease the synthesis and secretion of renal TGF-β1. In a prospective study, Campistol et al. [7] demonstrated that losartan reduces TGF-β1 plasma levels in kidney transplant patients with chronic allograft nephropathy. Captopril reduced TGF-β1 serum levels of 58 of the 409 patients with type 1 diabetes and overt nephropathy participating in the Collaborative Study Captopril Trial. These changes in TGF-β1 levels were related to the course of DN [8].

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The aim of this study was to determine the effect of losartan treatment on TGF-β1 plasma levels and urinary albumin excretion (UAE) in patients with type 2 diabetes mellitus, mild hypertension and microalbuminuria.

Patients and methods

Patients

Fourteen out-patients at the Hospital Clinic in Barcelona with type 2 diabetes mellitus (eight men; mean age 55±6 years; mean diabetes duration 12±7 years) were included in the study. All patients fulfilled the criteria for type 2 diabetes of the Expert Committee on Diagnosis and Classification of Diabetes Mellitus [9].

The patients studied took part in an open, uncontrolled, multicentre, study designed to evaluate the effect of two possible alternatives of treatment with losartan on UAE and blood pressure. The inclusion criteria were: type 2 diabetes mellitus. UAE between 20 and 200 μg/min and mild hypertension without treatment or on monotherapy (captopril or doxazosin).

The protocol was approved by the local ethics committees and informed consent was obtained from all the patients.

TGF-β1 plasma levels from 10 previously studied, healthy subjects from the laboratory (mean age 41±10 years) were used for comparison with diabetic subjects.

Study design

All the patients who fulfilled the selection criteria (visit 0) began a 4-week washout period for patients treated with ACE inhibitors or angiotensin II receptor antagonists, and a 2-week washout period was undertaken by patients treated with other antihypertensive drugs, during which no antihypertensive drugs were allowed. Patients with levels of systolic BP between 130 and 159 mmHg and diastolic BP between 85 and 99 mmHg after the washout period (visit 1) began a first 4-week treatment period with losartan at 50 mg once a day. After these 4 weeks of treatment (visit 2), patients achieving BP ≤130/85 mmHg continued with the same treatment for the following 4 weeks, while the patients who had higher BP levels were randomized to receive losartan 100 mg once a day or losartan 50 mg plus 12.5 mg of hydrochlorothiazide once a day. At the end of this period, the patients were re-evaluated (visit 3).

As a part of the multicentre study design, physical examination and office BP were performed on each visit. On appointments 1, 2 and 3, a 24-h urine collection was carried out to determine UAE, and fasting blood samples were obtained to measure glucose, HbA1c, and renal function. In addition, TGF-β1 plasma levels and 24-h ambulatory BP monitoring (ABPM) were measured on visits 1, 2 and 3.

Analytical methods and examinations

To determine TGF-β1 plasma levels, peripheral venous blood was obtained with EDTA as anticoagulant and was centrifuged at 1000 g for 30 min at 4 °C. The plasma was isolated and stored at −70 °C until the assay was performed. To activate latent TGF-β1 into immunoreactive TGF-β1 detectable by the immunoassay test, acidification (to a 0.1 ml sample add 0.1 ml of 2.5 N acetic acid/10 M urea) and neutralization (adding 0.1 ml of 2.7 N NaOH/1 M HEPES) procedures (pH 7.2–7.6) were carried out. TGF-β1 was determined using a solid-phase TGF-β1-specific sandwich enzyme-linked immunosorbent assay (ELISA: Quantikine: R&D Systems, Minneapolis, MN). A standard TGF-β1 curve was constructed using 2000, 1000, 500, 250, 62.5 and 31.5 pg/ml of recombinant human TGF-β1 protein. The minimum detectable level of TGF-β1 with the test was 7 pg/ml.

HbA1c was determined by high-speed liquid chromatography based on an ion exchange method.

Office BP (mean of three determinations) was measured according to the recommendations of the British Society of Hypertension, by a random zero sphygmomanometer with the subject in a sitting position for at least 5 min. ABPM was performed with the Spacelabs device (90207 model).

Statistical analysis

Data are given as mean ± SD. Data were analysed using the Wilcoxon signed-rank test. Linear correlations were assessed with Spearman’s correlation. A P-value (two-tailed)<0.05 was considered statistically significant.

Results

Losartan treatment was associated with a significant reduction in BP, UAE and TGF-β1, as summarized in Table 1. After the first 4 weeks of treatment with losartan, we observed a significant reduction in UAE which persisted until the end of the study. Basal TGF-β1 plasma levels decreased from 5.5±4.5 to 2.0±0.6 and 2.6±1.0 ng/ml after 4 and 8 weeks of treatment, respectively. Individual changes in TGF-β1 plasma levels are shown in Figure 1.

TGF-β1 plasma levels in the diabetic patients were higher compared with healthy subjects (5.5±4.5 vs 3.0±1.1 ng/ml, P<0.05). No differences were observed between baseline TGF-β1 plasma levels and the evolution of this cytokine or UAE in the whole group during the study. However, when we stratified the patients by baseline TGF-β1, seven showed TGF-β1 plasma levels higher than the mean plus one standard deviation of the mean observed in the control group. In relation to baseline values, at 4 and 8 weeks of treatment an important reduction was found in TGF-β1 plasma levels (from 9.0±3.9 to 2.1±0.7 and 2.5±0.7 ng/ml, respectively, P<0.01) and UAE (from 106±83 to 49±42 and 38±26 μg/min, respectively, P<0.05), with a good correlation between the percentage reduction of both parameters (r=0.83, P<0.01) (Figure 2). In the other seven patients, with normal baseline TGF-β1 plasma levels, we did not observe any change in TGF-β1 plasma levels (from 2.0±0.5 to 1.9±0.5 and 2.6±1.2 ng/ml, respectively) and UAE (from 87±45 to 70±76 and 90±51 μg/min, respectively) or any correlation in the evolution of both parameters, after treatment. There were no differences between the two groups in mean baseline ABPM (99±8 vs 97±5 mmHg) or in the percentage
Table 1. Ambulatory BP monitoring, TGF-β1 and urinary albumin excretion (UAE) at baseline, and 4 and 8 weeks after treatment

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>4 weeks</th>
<th>8 weeks</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-h SBP (mmHg)</td>
<td>136 ± 9</td>
<td>129 ± 9</td>
<td>130 ± 10</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>24-h DBP (mmHg)</td>
<td>77 ± 9</td>
<td>74 ± 8</td>
<td>74 ± 7</td>
<td>&lt;0.003</td>
</tr>
<tr>
<td>TGF-β1 (ng/ml)</td>
<td>5.5 ± 4.5</td>
<td>2.0 ± 0.6</td>
<td>2.6 ± 1.0</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>UAE (µg/min)</td>
<td>96 ± 65</td>
<td>59 ± 59</td>
<td>64 ± 47</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Mean ± SD. SBP, systolic blood pressure; DBP, diastolic blood pressure.

Fig. 1. Individual TGF-β1 plasma levels at baseline and at 4 and 8 weeks of treatment with losartan.

Fig. 2. Correlation between percentage change in TGF-β1 plasma levels and percentage change in urinary albumin excretion (UAE) in seven patients with basal TGF-β1 plasma levels higher than the mean plus one standard deviation of the mean. The correlation coefficient is 0.83 (P<0.01).

reduction observed at 4 weeks (3.9 ± 6.2 vs −4.1 ± 5.2%) and 8 weeks (−6.4 ± 5.9 vs −1.0 ± 6.9%) after treatment.

In the last 4 weeks of the study, five patients received treatment with losartan 100 mg/day, six patients received losartan 50 mg plus 12.5 mg of hydrochlorothiazide/day and three patients received losartan 50 mg/day. We did not observe any difference in relation to the evolution of TGF-β1 plasma levels according to the type of treatment. Throughout the study, there were no changes in HbA1c plasma levels and renal parameters.

Discussion

Our substudy of 14 type 2 diabetic patients with mild hypertension and microalbuminuria enrolled in a multicentre study evaluated the effect of losartan treatment on UAE, and showed a reduction in both UAE and TGF-β1 levels after treatment.

The TGF-β family consists of ubiquitous cytokines that function in an autocrine or paracrine fashion to produce cell growth and extracellular matrix accumulation in many cell types. Our study, in which the diabetic group had a mean plasma level of TGF-β1 double that of the normal subjects, is consistent with other studies of type 2 diabetic patients [10–12].

Most information concerning diabetes and TGF-β1 has been obtained from experimental and clinical studies on DN. Thus, high-content glucose medium increases TGF-β mRNA and protein levels in cultured proximal tubular cells and glomerular epithelial and mesangial cells [13]. Moreover, overexpression of TGF-β1 in the glomeruli and tubulointerstitial in experimental and human diabetes has been reported [14,15]. Patients with DN present increased excretion of urinary TGF-β1, and a TGF-β1 gene mutation has been described recently in patients with type I diabetes and nephropathy [16]. All these data strongly support the hypothesis that TGF-β1 is involved in the development of diabetic glomerulosclerosis.

The interruption of the TGF-β1 system is an interesting strategy to treat DN progression [17]. Neutralizing anti-TGF-β1 antibodies reduce glomerular hypertrophy and attenuate the increase of extracellular matrix mRNA levels in streptozotocin-induced diabetic mice [6]. However, this form of inhibition of TGF-β1 activity is not applicable in chronic human diseases such as DN. Decorin is a glomerular proteoglycan that binds TGF-β1 and neutralizes its activity. Its use in experimental animal studies has provided different results depending on the target tissue evaluated. It has been useful in rats with glomerulonephritis to inhibit matrix deposition, but does not appear to have any beneficial effect on a caroty injury model in rats. This is probably because decorin is concentrated in the kidney where it achieves higher concentrations. The possible therapeutic use of decorin remains to be explored [4–6].

In animal models of various kidney diseases, several studies have shown that ACE inhibition attenuates progression of nephropathy with coincident reduction of glomerular TGF-β1 production [18,19]. In this respect, two clinical studies have been published recently. In the first study [7], the effect of treatment with 50 mg of losartan during 8 weeks on TGF-β1 plasma levels in transplant patients with chronic allograft nephropathy was studied prospectively. This study clearly demonstrated, for the first time, that an angiotensin II
receptor antagonist reduces TGF-β1 plasma levels in humans. In the second study, TGF-β1 was measured after 6 months of captopril or placebo treatment in a subset of type I diabetic patients enrolled in the Captopril Collaborative Study Group Trial, in which serum samples were available. In captopril-treated patients, there was a 14% reduction in TGF-β1 serum levels while in the placebo group the levels increased 11% [8]. Our study is the first in which the effect of an angiotensin II receptor antagonist on TGF-β1 plasma levels has been studied prospectively in patients with diabetes and nephropathy. The observation that losartan treatment produced a 64% mean reduction in TGF-β1 plasma levels strongly supports the hypothesis that one of the therapeutic effects of the angiotensin II receptor antagonists is the inhibition of TGF-β1 production through the renin-angiotensin system.

An interesting finding in our study, albeit uncontrolled and with a small number of patients, is that although losartan produces a similar BP reduction in all patients, the antiproteinuric effect of this drug only occurs in patients with increased plasma levels of TGF-β1. Thus, it may be speculated that TGF-β overproduction is not a universal pathogenic factor among patients with diabetic nephropathy. This could define two groups of patients with DN, one with normal TGF-β1 plasma levels in which other pathogenic factors may play a prominent role, and another in which TGF-β1 overproduction may be very important. A reduction in TGF-β1 plasma levels as a consequence of inhibition of the renin-angiotensin system may be a good therapeutic strategy in the second group but it may not be effective in the first group. This hypothesis agrees with the observation of Parving et al. [20] that the deletion polymorphism in the ACE gene reduces the long-term beneficial effect of captopril treatment in patients with type I diabetes and nephropathy. It would be very interesting to know whether patients with this deletion polymorphism present lower TGF-β1 plasma levels.

In summary, this pilot study suggests that losartan is a useful therapeutic strategy to decrease UAE in type 2 diabetic patients with nephropathy. Losartan reduces BP and also reduces TGF-β1 levels. These results suggest that patients with elevated TGF-β1 levels receive the greatest benefits from the blockade of angiotensin II action through the inhibition of ACE or the use of angiotensin II receptor antagonists. Therefore, the routine determination of TGF-β1 plasma levels in patients with DN may be useful to design the therapeutic strategy of type 2 diabetic patients with nephropathy.

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References