Effect of tranilast in early-stage diabetic nephropathy

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Abstract

Background. Tranilast is an antifibrotic drug known to suppress collagen synthesis by fibroblasts by interfering with the effects of TGF-β. We recently reported that it slowed the progression rate of advanced diabetic nephropathy (DN) by reducing the accumulation of collagens in renal tissue. The present study was undertaken to examine the effect of tranilast on early-stage DN.

Methods. Among out-patients with diabetes mellitus, we selected patients with (i) urinary albumin excretion of 30–1000 mg/g creatinine (gCr) in the first morning urine, (ii) serum creatinine (SCr)/C20 gCr ≤ 1.2 mg/dl and no haematuria and (iii) currently taking an angiotensin-converting enzyme inhibitor or angiotensin receptor blocker. Twenty patients fulfilled the criteria, of whom 10 were selected at random and commenced on tranilast [100 mg, 3 times daily; T(+) group]. The remaining 10 patients comprised the T(-) group.

Excretion of both urinary type IV collagen (U-IV) and albumin (U-A) in the first morning urine was measured every 3 months. The follow-up period was 1 year.

Results. At baseline, no significant differences were observed in SCr, HbA1c, blood pressure and U-A excretion between the T(+) and T(-) groups, but U-IV excretion in the T(+) group was higher than in the T(-) group (6.4 ± 0.66 vs 3.7 ± 0.36 mg/gCr, mean ± SEM, P < 0.01). At 1 year, SCr was not different from the baseline in either group. In the T(+) group, however, excretion rates of both U-IV and U-A tended to decrease with time, and after 1 year, were significantly decreased compared with excretion at baseline (U-A: 279 ± 78 to 191 ± 62 mg/gCr; P = 0.049, U-IV: 6.4 ± 0.66 to 4.4 ± 0.99 µg/gCr; P = 0.02). In contrast, in the T(-) group, excretion of both U-A and U-IV tended to increase with time. The changes of both U-A and U-IV excretions in the two groups took statistically different trends through tranilast treatment (P = 0.01 and P = 0.04, respectively).

Conclusions. Our results suggest that tranilast could be therapeutically beneficial in early-stage DN.

Keywords: albuminuria; collagen; diabetic nephropathy; extracellular matrix; tranilast

Introduction

Worldwide, the number of patients requiring haemodialysis for diabetic nephropathy (DN) has increased, and this has become a serious management problem [1,2]. Several factors contribute to the progression of DN, including poor glycaemic control [3], hyper tension [4], proteinuria [5], lipid abnormalities [6] and genetic anomalies [7,8]. The progression of DN is closely related to the accumulation of extracellular matrix in renal tissue [9], and the major component of the matrix is a collagen synthesized by various cells, including macrophages and fibroblasts [10,11].

Tranilast [N-(3, 4-dimethoxycinnamoyl) anthranilic acid] is an antifibrotic agent that inhibits transforming growth factor-β (TGF-β) release from various cells, including fibroblasts and macrophages, leading to reduced collagen synthesis by fibroblasts [12,13]. We recently reported that tranilast slowed the rate of decline of renal function in patients with advanced DN with a non-significant trend towards reduction in urinary protein and type IV collagen excretion [14]. In the present study, we examined the effect of tranilast on early-stage DN.

Patients and methods

Patients

Between April 2002 and October 2002, we selected patients who fulfilled the following criteria from among out-patients with diabetes mellitus: (i) values of urinary albumin excretion of 30–1000 mg/g creatinine (gCr) in the first morning urine without haematuria (urinary red blood cells < 5/high-powered field in urinary sediment), (ii) serum creatinine
(SCr) <1.2 mg/dl and (iii) taking an angiotensin-converting enzyme inhibitor (ACEi) or angiotensin II receptor blocker (ARB). Twenty patients were selected for the present study, and the patients were all type 2 diabetic. These patients were divided into two groups at random: the T(+) group (n = 10) comprised patients who were treated with tranilast (100 mg/day, 3 times daily), and the T(−) group (n = 10) comprised the remaining patients who were not treated with tranilast. The study was approved by our institutional ethics committee, and each patient gave informed consent. The follow-up period was 1 year. Doses of ACEi or ARB and other antihypertensive agents were not modified during the study.

**Laboratory parameters**

Urinary albumin excretion (U-A) and urinary type IV collagen (U-IV) excretion in the first morning urine were measured just before commencement of tranilast therapy (baseline) and every 3 months during the study. SCr and HbA1c were also measured at the same time intervals. The values of U-A and U-IV in the first morning urine were measured using a latex turbidimetric immunoassay kit (Eiken Chemical Industries, Tokyo, Japan) [15] and a sandwich EIA kit (Fuji Chemical Industries, Toyama, Japan) [16], respectively. Values for U-A and U-IV in the first morning urine were divided by urinary creatinine concentrations to exclude the influence of urine concentration. Normal ranges were <10 mg/gCr and <4.9 μg/gCr, respectively.

**Statistical analysis**

All values were expressed as mean ± SEM. The primary end point was defined as chronological change in values of U-IV during the course of the study. Because the distribution of albuminuria was highly skewed, values for U-A excretion were log-transformed before subsequent statistical analysis. Statistical analyses were performed using the paired or unpaired t-test, one-factor ANOVA and repeated-measure ANOVA where appropriate. P < 0.05 was considered significant.

**Results**

**Clinical and laboratory findings at baseline and final follow-up**

No patients dropped out during the study, and no symptoms that were considered side effects of tranilast were observed in the T(+) group.

At baseline, patients of the T(+) group were older than those of the T(−) group. However, age, the percentages of patients with diabetic retinopathy and those treated with insulin were comparable between the two groups. The numbers of patients treated with ACEi and ARB were two and eight, respectively, in each group (Table 1). Clinical data of patients at baseline showed no differences in systolic and diastolic blood pressures, HbA1c, SCr and U-A excretion between the two groups. Only U-IV excretion was significantly higher in the T(+) group than in the T(−) group (P < 0.01; Table 2).

In the T(+) group, excretion rates of both U-A and U-IV at final follow-up were significantly decreased compared with those at baseline (P = 0.049 and P = 0.02, respectively; Table 2 and Figure 1). In the T(−) group, on the other hand, values of both U-A and U-IV excretion at final follow-up were higher than those at baseline, but the differences were not significant (Table 2). The values of systolic and diastolic

**Table 1. Characteristics of participating patients**

<table>
<thead>
<tr>
<th>Agea (years)</th>
<th>Gender (M/F)</th>
<th>Retinopathyb</th>
<th>Insulinc</th>
<th>ACEi/ARBd</th>
</tr>
</thead>
<tbody>
<tr>
<td>T(+) group (n = 10)</td>
<td>73 ± 2</td>
<td>2/8</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>T(−) group (n = 10)</td>
<td>70 ± 3</td>
<td>4/6</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

aData are mean ± SEM. 
bNumber of patients with retinopathy. 
cNumber of patients treated with insulin. 
dNumber of patients treated with angiotensin-converting enzyme inhibitor or angiotensin II receptor blocker.

**Table 2. Clinical data of patients at baseline and final follow-up**

<table>
<thead>
<tr>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
<th>HbA1c (%)</th>
<th>SCr (mg/dl)</th>
<th>U-A (mg/gCr)</th>
<th>U-IV (μg/gCr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T(+) group (n = 10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>131 ± 3.0</td>
<td>76 ± 2.7</td>
<td>7.2 ± 0.25</td>
<td>0.91 ± 0.04</td>
<td>279 ± 78</td>
</tr>
<tr>
<td>Final</td>
<td>135 ± 2.7</td>
<td>80 ± 2.5</td>
<td>7.1 ± 0.16</td>
<td>0.94 ± 0.05</td>
<td>191 ± 62***</td>
</tr>
<tr>
<td>T(−) group (n = 10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>139 ± 3.2</td>
<td>76 ± 2.8</td>
<td>7.2 ± 0.26</td>
<td>0.87 ± 0.06</td>
<td>290 ± 130</td>
</tr>
<tr>
<td>Final</td>
<td>133 ± 3.1</td>
<td>80 ± 3.0</td>
<td>6.9 ± 0.18</td>
<td>0.91 ± 0.06</td>
<td>486 ± 270</td>
</tr>
</tbody>
</table>

Data are mean ± SEM. 
*P < 0.01 vs T(−) at baseline; **P = 0.02 vs T(+) group at baseline; ***P = 0.049 vs T(+) group at baseline. 
SBP, systolic blood pressure; DBP, diastolic blood pressure; SCr, serum creatinine; U-A, urinary albumin excretion; U-IV, urinary type IV collagen excretion.
blood pressures, SCr and HbA1c at final follow-up were statistically comparable with those at baseline.

**Chronological changes in U-A and U-IV excretion**

Figure 2 shows the serial changes in U-A and U-IV excretions measured in the first morning urine every 3 months. Although U-A excretion in the T(+) group at final follow-up was significantly decreased compared with that at baseline, as mentioned above (Table 2, Figure 1A), the time trend of the decrease was not significant by one-factor ANOVA (Figure 2A). The time trend of increase in the T(−) group was also not significant. The result of U-IV excretion by one-factor ANOVA was similar to that of U-A excretion (Figure 2B).

However, when the group data were analysed by repeated-measure ANOVA, there were significant differences in the serial changes of both U-A and U-IV excretions between the T(+) and T(−) groups ($P=0.01$ and $P=0.04$, respectively; Figure 2). These findings indicate that the changes of U-A and U-IV excretions in the two groups took a different trend through tranilast treatment.

**Discussion**

The present study demonstrates that treatment with tranilast seems to decrease U-A and U-IV excretion in
patients with DN at the early stage of diabetes mellitus. This indicates that treatment with tranilast is a potentially useful therapy for suppressing the progression of early DN.

We recently reported that treatment with tranilast slowed the rate of the progression of advanced DN (mean SCr and U-IV excretion rates of studied patients at baseline were 2.96 mg/dl and 32.6 μg/gCr, respectively) [14]. The mean of the 1/SCr slope during tranilast treatment was nearly half that observed before treatment. However, all patients in the study showed a continuous decline in renal function despite the treatment. Therefore, we decided to analyse the effect of tranilast on earlier stages of DN, and thus undertook the present study.

Tranilast is an antifibrotic agent, and is used clinically for the treatment of keloid formation after skin injury [13] and scleroderma [17]. Although the precise mechanisms underlying the antifibrotic effects of tranilast are not completely understood, tranilast has been shown to inhibit, in particular, TGF-β release from a range of cells, including skin fibroblasts [12,17], cardiac fibroblasts [18], vascular smooth muscle cells [19–21], Tenon’s capsule and corneal stromal fibroblasts [22], hepatic stellate cells [23] and renal interstitial fibroblasts [24]. TGF-β has an important role in the synthesis and accumulation of extracellular matrix, leading to fibrosis, including renal fibrosis [25,26]. The main component of extracellular matrix is collagen, and an increase in mesangial type IV collagen has been reported to participate in diabetic glomerulosclerosis [9–11].

To determine the effect of tranilast, we investigated the excretion of U-A and U-IV, which have been reported to be useful markers for DN [27–30]. Excretion of both U-A and U-IV tended to decrease in the T(+) group and increase in the T(−) group, although the difference was not significant. However, time trends of U-IV excretion in the T(+) and T(−) groups showed chronologically differential movements (by repeated-measure ANOVA). These results emphasize that tranilast treatment may produce a beneficial effect on the progression of DN. The effects of tranilast on the kidney have also been reported in several experimental studies, where tranilast reduced interstitial fibrosis, tubular atrophy, glomerulosclerosis and proteinuria in the streptozotocin-induced diabetes model [24,31], unilateral ureteric obstruction model [32] and remnant kidney model [33]. In particular, Mifsud et al. [24] reported that, in connection with the present study, increased type IV collagen in the peritubular basement membrane was significantly decreased by tranilast treatment in streptozotocin-induced diabetic rats.

In addition to the suppression of collagen synthesis by fibroblasts, tranilast suppresses the production and release of platelet-derived growth factor (PDGF) and interleukin-1 (IL-1) by monocytes/macrophages, leading to suppression of migration and proliferation of smooth muscle cells [12,19–21]. Tranilast also inhibits the expression of monocyte chemoattractant protein-1 (MCP-1) in rat mesangial cells through suppression of nuclear factor-κB (NF-κB) [34]. Monocytes and macrophages, which are recruited by MCP-1, have been reported to participate in the progression of DN [36,37]. In experimental diabetic rats, advanced glycation end-products (AGEs) are involved in the overproduction of PDGF and IL-1 by mesangial cells or monocytes/macrophages [38–40], and in overproduction of MCP-1 by podocytes [41], thereby contributing to glomerulosclerosis and interstitial fibrosis. These known actions of tranilast potentially explain a trend towards decrease in U-IV excretion in the present study.

Although several factors, such as poor glycaemic control, hypertension and lipid abnormalities, can be improved in patients with DN, a gradual decline in renal function is observed in a significant proportion of patients. With regard to antihypertensive agents, ACEi and ARB are currently essential elements of treatment for patients with DN. ACEi and ARB reduce glomerular capillary hypertension and glomerular hypertrophy, and reduce renal functional losses [42–44]. All patients in the present study were treated with ACEi or ARB before and during tranilast treatment. The aforementioned immunological effects of tranilast, when added to the haemodynamic effect of ACEi or ARB, may confer a more favourable prognosis on patients with DN. However, one patient in the T(+) group showed a chronological increase in both U-A and U-IV excretions (Figure 1). The patient showed the poorest glycaemic control among patients of the study (HbA1c of 8.1% at baseline and 7.8% at final follow-up). Poor glycaemic control may weaken the effect of tranilast on DN.

In summary, we report here that tranilast treatment may suppress accumulation of collagen in renal tissue and may be therapeutically useful for early-stage DN, in addition to our reported beneficial effects of the same agent in advanced DN [14]. However, sample size of the study is very small. A prospective controlled study in a larger number of patients and longer observation may reveal a more clear effect of tranilast on DN.

References
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