Inhibitory Effect of Tranilast on Hypertrophic Collagen Production in the Spontaneously Hypertensive Rat Heart

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ABSTRACT—Tranilast, N-(3,4-dimethoxycinnamoyl) anthranilic acid, a widely used antiallergy drug in Japan, has been shown to inhibit transforming growth factor-β1 release from fibroblasts and reduce collagen synthesis in keloid cells. In the present study, we have investigated the effect of this drug on cardiac hypertrophy in spontaneously hypertensive rats (SHR), with a focus on the cardiac collagen matrix, which is associated with myocardial stiffness. Twenty-four-week-old SHRs and Wistar Kyoto rats (WKYs) were administered tranilast (300 mg/kg) orally once a day for 4 weeks. This treatment significantly suppressed increases in left ventricular collagen concentration (P < 0.05) and the left ventricular weight/body weights ratios (P < 0.05) in SHRs, and tranilast was ineffective on collagen concentration and ventricular weight/body weights ratios in WKYs. Tranilast did not affect systolic or diastolic blood pressure, end-diastolic left ventricular pressure and heart rate in both SHRs and WKYs, and the agent did not change positive dp/dt of cardiac output in SHRs. The pressure-volume relationship curve was shifted to the left by the drug; the slope (k) of the logarithm of the pressure-volume relationship curve was significantly increased (P < 0.05) in SHRs. It is concluded that the suppression of increases in cardiac collagen and left ventricular mass by tranilast results in a corresponding prevention of cardiac stiffness as studied in the SHR.

Keywords: Left ventricular hypertrophy, Tranilast, Cardiac collagen, Myocardial stiffness, Spontaneously hypertensive rat

Left ventricular hypertrophy is a major adaptative response of the heart to chronic hemodynamic overload. However, it is becoming increasingly clear that left ventricular hypertrophy is an important independent risk factor for development of long-term cardiovascular complications (1–3). One third of all patients with symptomatic heart failure have diastolic dysfunction of their left heart chamber while systolic function is preserved (4–6). It has been suggested that alterations in the cardiac interstitium may contribute to changes in diastolic function of hypertrophied heart (7). It has also been suggested that myocardial fibrosis may restrict myofibrillar motion and thereby impair overall cardiac function (8).

Currently, angiotensin II has been reported to have an important regulatory role on cardiac fibroblast function (9, 10); it stimulates synthesis of collagen from cardiac fibroblasts directly through angiotensin II type 1 receptors (9) or by promoting the release of transforming growth factor (TGF)-β1, which stimulates synthesis of collagen from cardiac fibroblasts (11, 12).

Tranilast, N-(3,4-dimethoxycinnamoyl) anthranilic acid, has been widely used in patients with allergy in Japan (13, 14). It has inhibited the release of TGF-β1 from fibroblasts and reduced collagen synthesis in the keloid (15). Further, in a double-blind, large-scale multicentre trial, tranilast reduced the rate of post-percutaneous transluminal coronary angioplasty restenosis (16). In the light of these reports, the aim of this study is to investigate its effect on cardiac hypertrophy in spontaneously hypertensive rats (SHRs) and Wistar Kyoto rats (WKYs), in particular its action on the cardiac collagen matrix. Furthermore, we studied if a reduction in cardiac collagen matrix would reduce myocardial stiffness.

MATERIALS AND METHODS

Surgical procedures

Male SHRs and WKYs, 23-week-old, from Charles River (Yokohama) were allowed to adjust to laboratory conditions for one week before the experiments. In this study, 9 24-week-old SHRs were orally administered tranilast (at a dose of 300 mg/kg), dissolved by 0.5%
carboxymethyl cellulose (CMC), once a day for 4 weeks, and another 9 SHRs were administered with 0.5% CMC alone for 4 weeks. Tranilast or 0.5% CMC was administered in the same manner to 2 groups (10 rats per group) of WKYs.

Under 1.5% halothane anesthesia with 30% oxygen, 70% room air and spontaneous breathing, a catheter for measuring systolic and diastolic aortic blood pressure, heart rate, end-diastolic left ventricular pressure and dp/dt was inserted into the right carotid artery with the other end connected to a pressure transducer (MPU-05, RP-5; Nihon Kohden, Tokyo). Under artificial ventilation, the left chest was opened and the probe of an electromagnetic flowmeter (Nihon Kohden) was attached to the ascending aorta for measuring cardiac output.

The pressure-volume relationship in the left ventricle in SHR was determined after diastolic arrest of the heart with potassium chloride. A double lumen catheter was advanced into the left ventricular cavity via the aorta and then secured by a ligature around the atrio-ventricular groove. The right ventricle was incised to eliminate any compressive effect. Saline was infused at a rate of 0.34 ml/min, and pressure was simultaneously recorded over 0–30 mmHg. An index of left ventricular chamber stiffness, k, was calculated as the slope of the logarithm of pressure versus volume relationship over the pressure range of 2.5–30 mmHg.

**Determination of perivascular collagen areas**

After measuring the pressure-volume relationship, the left ventricle was fixed with 2% glutaraldehyde and 1% paraformaldehyde in by 0.1 M phosphate buffer (pH 7.4), embedded in paraffin and the myocardial tissue was examined microscopically after Azan staining. The stain facilitates morphometry by permitting a clear distinction between connective tissue (blue) and muscle fibers (red). The areas of the perivascular collagen normalized to the vessel luminal area of intramural coronary arteries were determined by using an automated image analyzer (Videoplan, Oberkochen, Germany). Only those intramyocardial vessels that appeared circular on the cross-section were analyzed; on the average, there were 7 such vessels found in the left ventricle. The investigator responsible for the morphometrical analysis was unaware of the treatment given each experimental group.

**Statistical analyses**

Data were represented as means±S.E. Statistical analyses were done by ANOVA followed by the Bonferroni/Dunn test. For the pressure-volume relationship, a two-way ANOVA was performed. P<0.05 was considered significant.

**RESULTS**

Tranilast at a dose of 300 mg/kg per day for 4 weeks did not affect animal body weight, systolic or diastolic blood pressure and heart rate in both SHRs and WKYs (Table 1). Furthermore, in SHRs, end-diastolic left ventricular pressure, positive dp/dt and cardiac output were not affected by tranilast treatment (Table 2).

The left ventricular weight / body weight ratio and hydroxyproline contents in SHRs were about 30% and 44% greater than those in WKYs, respectively. Treatment with tranilast slightly but significantly (P<0.05) reduced the left ventricular weight / body weight ratio (Fig. 1), and the hydroxyproline contents was significantly (P<0.001) decreased by tranilast treatment in SHRs (Fig. 2). However, tranilast did not affect either the left ventricular weight / body weight ratio or hydroxyproline contents in WKYs (Figs. 1 and 2).

The pressure-volume relationship curve in SHRs treated with tranilast was significantly (P<0.05) shifted to the left compared with that in SHRs treated without tranilast (Fig. 3). The slope (k) of the logarithm of the pressure-versus-volume relationship in SHRs treated with tranilast significantly (P<0.05) increased compared with that in SHRs treated without tranilast (Table 3).

In histological examinations, perivascular fibrosis of intramural coronary arteries was present (Fig. 4). The perivascular collagen area normalized to vessel luminal
Table 1. Body weight and hemodynamics in 28-week-old SHRs and WKYs

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SHR</th>
<th>WKY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle 9</td>
<td>Tranilast 9</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>391±6</td>
<td>406±5</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>160±4</td>
<td>151±3</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>124±4</td>
<td>115±3</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>317±8</td>
<td>304±3</td>
</tr>
</tbody>
</table>

Data each represent a mean±S.E.

Table 2. Hemodynamics in 28-week-old SHRs

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SHR</th>
<th>WKY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle 9</td>
<td>Tranilast 9</td>
</tr>
<tr>
<td>Left ventricle dp/dt_{max} (mmHg/sec)</td>
<td>4530±518</td>
<td>4333±187</td>
</tr>
<tr>
<td>End-diastolic left ventricular pressure (mmHg)</td>
<td>6.21±0.90</td>
<td>5.67±0.63</td>
</tr>
<tr>
<td>Cardiac output (ml/min)</td>
<td>114±1</td>
<td>111±1</td>
</tr>
</tbody>
</table>

Data each represent a mean±S.E.

Fig. 1. Effect of tranilast on left ventricular weight / body weight ratio. Data represent the mean±S.E. of 9 SHRs and 10 WKYs treated or untreated by tranilast. LV: left ventricle. ***P<0.001 vs SHRs untreated with tranilast.

area of the left ventricle was significantly (P<0.01) reduced in animals treated with tranilast compared with that in untreated animals (Fig. 5). There were no differences in the diameters of arteries, which were used for measurement of collagen area ratio, between values obtained from animals treated with tranilast and untreated animals (168±74 and 166±73 µm, respectively).

Fig. 2. Effect of tranilast on left ventricular hydroxyproline concentration. Data represent the mean±S.E. SHRs treated (9 animals) or untreated (7 animals) by tranilast and WKYs treated with (10 animals) or without (10 animals) tranilast were evaluated. LV: left ventricle. ***P<0.001 vs SHRs untreated with tranilast.

DISCUSSION

In this study, using the SHR as a model, we examined the effect of the antiallergic drug tranilast on collagen formation in myocardial tissue. The treatment reduced
myocardial collagen contents, and this resulted in a marked prevention of myocardial stiffness. Likewise, the drug inhibited the increase in the areas of perivascular collagen in the coronary artery.

Clinical and experimental observations suggest that the renin-angiotensin system has an important role in the development of left ventricular hypertrophy (9, 10, 17–19), particularly in pressure-overload cardiac hypertrophy. It is believed that the renin-angiotensin system is associated with accumulation of collagen in the extracellular matrix as well as with an increase in the size of myocytes (20). Recently, it has been reported that rat cardiac fibroblasts expressed angiotensin II type 1 receptor, and angiotensin II increased the synthesis of extracellular matrix proteins including collagen via angiotensin II type 1 receptors (9). It is hypothesized that angiotensin II increases the synthesis of extracellular matrix directly or by triggering the release of TGF-β1, from fibroblasts (9, 11, 12). Norton et al. (21) demonstrated that treatment with an angiotensin converting enzyme, captopril, for 22 weeks in SHRs reduced cardiac hydroxyproline contents to the same values as those in WKYs. Cardiac hydroxyproline contents in SHRs were about 30% more than those in the same aged WKYs. In this study, hydroxyproline contents in SHR was also about 44% higher than that in the same aged WKYs and tranilast reduced hydroxyproline contents by 7% compared with those in SHRs treated without tranilast, suggesting that efficacy of tranilast in reduction of collagen contents is less than that of captopril. Grover-McKay et al. (22) reported that hydroxyproline contents in 22-week-old SHRs increased by 54% for 11 weeks. In this study, hydroxyproline contents in 24-week-old SHRs increased by 13% for 4 weeks (data not shown).

Although angiotensin II may play a key role in myocardial fibrosis, numerous factors, including TGF-β1, connective tissue growth factor, mast cells (23), free radicals (24) and endothelin may be related to myocardial fibrosis. It has been reported that mast cells and free radicals may play a part in the development of the myocardial fibrosis in hypertensive cardiac hypertrophy. Although tranilast also stabilizes mast cells and inhibits radical generation, it is unclear that these actions by tranilast may be attributable to reduction of myocardial fibrosis in this study.

The dosages of tranilast used in this study are about the same as the effective dosage used in the experimental allergic studies and the same as the plasma concentrations used in humans. In this study, tranilast reduced the left ventricular weight per body weight ratio. Although, it is unclear whether tranilast has a direct effect to suppress hypertrophic myocytes or not, Kikuchi et al. (25) reported that tranilast inhibited the proliferation of the cultured smooth muscle cells induced by several growth factors, and tranilast also blocks angiotensin II AT1 receptors (26). These findings suggest that tranilast may have an inhibitory effect on the progression of hypertrophic myocytes.

The first manifestation of hypertensive cardiopathy is a diastolic dysfunction, a major factor in chronic heart failure. Alteration of diastolic function alone is responsible for failure in almost 40% of the cases (27–29). It has been reported that in an experimental model of cardiac hypertrophy, abnormalities in myocardial perivascular and interstitial fibrosis were prevented by the use of inhibitors of collagen crosslinking (30). The reduction in

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**Table 3.** The slope (k) of the logarithm of the pressure-versus-volume relationship

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>The slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5% CMC</td>
<td>9</td>
<td>2.158±0.142</td>
</tr>
<tr>
<td>Tranilast</td>
<td>9</td>
<td>1.893±0.036*</td>
</tr>
</tbody>
</table>

Data each represent a mean±S.E. *P<0.05 vs animals treated with 0.5% CMC.
Fig. 4. Micrographs of the coronary artery and perivascular collagen. a: control, b: an animal treated with tranilast. The coronary artery wall thickening and an increase in perivascular collagen observed in the control group were greater than those in animals treated with tranilast. Arrows indicate the perivascular collagen fiber area. Azan stain. × 50.
cardiac collagen formation observed in this study in the presence of tranilast is in line with the above reports.

In conclusion, during this experimental period, the collagen concentration in the left ventricle and the left ventricular weight were increasing, and these may result in the impairment of cardiac stiffness. The inhibition of the increase in cardiac collagen and the reduction of ventricular weight by tranilast may result in a corresponding prevention of abnormal cardiac stiffness.

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