Dosing Time-Dependent Effect of Trandolapril on the Prevention of Cardiac Hypertrophy in Rats With Aortic Banding

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ABSTRACT—Trandolapril was given to male Wistar rats with aortic banding at 10 AM or 10 PM for 6 weeks to examine the influence of dosing time on the development of left ventricular mass (LVM). Aortic banding increased the LVM compared with the sham-operated animals (P<0.01). Trandolapril (1 mg/kg) at 10 AM reduced LVM (1.74 ± 0.04 [S.E.M.] mg/g) more than the dosing at 10 PM (1.92 ± 0.04 mg/g, P<0.05), suggesting that trandolapril has a dosing time-dependent effect in the prevention of cardiac hypertrophy in rats with aortic banding.

Keywords: Cardiac hypertrophy, Dosing time, Trandolapril

Dosing time-dependent changes in the effect of antihypertensive drugs are reported in hypertensive patients (1). Such a chronopharmacological approach may have different preventive effect against hypertensive organ damage. For example, we previously showed that nitrendipine, a calcium channel blocker, has a dosing time-dependent preventive effect against the development of cardiac hypertrophy in spontaneously hypertensive rats (SHR) (2). In that study, nitrendipine dosed at 10 AM (early phase of the resting period) did not affect ventricular weight, whereas a significant reduction in this parameter was observed in rats dosed at 10 PM (early phase of the active period). The precise mechanism of this chronopharmacological phenomenon is unclear, but the dosing time-dependent change in the pharmacokinetic profiles may be involved (3).

Angiotensin-converting enzyme (ACE) inhibitor, another category of antihypertensive agent, is also reported to have the dosing time-dependent effect on the daily profile of blood pressure (4, 5). Duration of antihypertensive effects of ACE inhibitors are greater after dosing at evening than after dosing in the morning. These data led us to speculate that the preventive effect of an ACE inhibitor against cardiac hypertrophy is greater after dosing in the evening in hypertensive patients. The present study was the first step to address this issue using hypertensive rats. Trandolapril, an ACE inhibitor, was given once daily for six weeks at different times to rats with abdominal aortic banding, which develops cardiac hypertrophy due to hemodynamic load and enhanced renin-angiotensin system. Dosing time-dependent changes in the effect of trandolapril on left ventricular (LV) hypertrophy and inhibition of serum ACE activity were examined.

Male Wistar rats (Japan SLC, Shizuoka) weighing 120 to 140 g were housed in a specific pathogen-free room with a 12-h light/dark cycle (7 AM light on and 7 PM light off). They were given standard rat chow (CE-2; Japan Clea Co., Ltd., Tokyo) and water. After 14 days of acclimatization period, rats were divided into six groups as follows:

1) controls, sham-operated (n = 9)
2) aortic banding + vehicle (n = 11)
3) aortic banding + trandolapril 0.01 mg/kg per day at 10 AM (n = 11)
4) aortic banding + trandolapril 0.01 mg/kg per day at 10 PM (n = 11)
5) aortic banding + trandolapril 1.0 mg/kg per day at 10 AM (n = 11)
6) aortic banding + trandolapril 1.0 mg/kg per day at 10 PM (n = 11)

For aortic banding, rats were anesthetized with pentobarbital sodium (50 mg/kg, intraperitoneal). The abdominal aorta was exposed above the left renal artery and a 3-0 silk thread passed beneath it. A blunted injection needle (0.7-mm outer diameter) was placed alongside the aorta. After aorta and needle was tied, the needle was removed. Control rats were subjected to the same procedure except for aortic banding. The study was conducted according to Use and Care of Experimental Animals Committee of Jichi Medical School.

Drug treatment was initiated at the day after operation
and continued for 6 weeks. Vehicle (0.5% methyl cellulose) was given twice daily (10 AM and 10 PM) by gavage to the aortic banding rats. Trandolapril suspended in a 0.5% methyl cellulose solution was given once daily by gavage to the respective groups. Dose was adjusted by body weight determined twice a week.

Six weeks later, rats were anesthetized with pentobarbital sodium (50 mg/kg, intraperitoneal), and a polyethylene catheter was inserted into the right common carotid artery to measure mean blood pressure. The measurement of blood pressure was performed at 22 to 26 h after the final dose of trandolapril. Blood pressure in the vehicle-treated rats and sham-operated rats was recorded at 8 to 12 AM. After blood pressure was recorded, blood was collected through the catheter for assay of serum ACE activity. The heart was then excised, cleaned of blood with saline, and gently blotted to dryness, and LV weight including septum was measured. LV weight was normalized for body weight. Serum ACE activity was determined by colorimetry (6).

Data are shown as means ± S.E.M. Statistical analyses were performed using one-way ANOVA. If significant difference was observed among groups, data of two groups were analyzed by the post hoc test as appropriate. A P less than 0.05 was considered significant.

Treatment with once-daily trandolapril decreased body weight in the aortic banding rats in a dose-dependent manner. This parameter in the rats treated with the low- or high-dose of trandolapril was not significantly different between the morning and evening trials (Table 1).

Arterial blood pressure measured through the common carotid artery was significantly higher in the vehicle-treated aortic banding rats (152.9 ± 3.9 mmHg) than in the sham-operated rats (113.3 ± 7.1 mmHg). A low-dose of trandolapril dosed in the evening, but not in the morning, significantly reduced blood pressure while there was not a statistical difference between the groups (131.3 ± 6.2 mmHg in the evening trial, 147.7 ± 6.7 mmHg in the morning trial, P>0.05). A high-dose of trandolapril further lowered blood pressure in the groups dosed in the morning (116.4 ± 7.2 mmHg) and evening (119.8 ± 5.9 mmHg) to a comparable level observed in the sham-operated rats (Fig. 1A).

Aortic banding induced the increase in LV mass (2.18 ±

### Table 1. Body weight, transstenotic pressure gradient and serum ACE activity in the aortic banding rats at the end of the treatment with trandolapril or vehicle for 6 weeks

<table>
<thead>
<tr>
<th></th>
<th>ML (n = 11)</th>
<th>EL (n = 11)</th>
<th>MH (n = 11)</th>
<th>EH (n = 11)</th>
<th>VHCL (n = 11)</th>
<th>SHAM (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>309 ± 6</td>
<td>304 ± 3</td>
<td>294 ± 4</td>
<td>277 ± 3</td>
<td>322 ± 5</td>
<td>319 ± 7</td>
</tr>
<tr>
<td>Serum ACE activity (U/L)</td>
<td>20.2 ± 0.5</td>
<td>18.5 ± 0.9</td>
<td>11.5 ± 0.6</td>
<td>12.0 ± 1.0</td>
<td>30.2 ± 1.1</td>
<td>32.4 ± 1.3</td>
</tr>
</tbody>
</table>

ML, 0.01 mg/kg trandolapril dosed at 10 AM; EL, 0.01 mg/kg trandolapril dosed at 10 PM; MH, 1.0 mg/kg trandolapril dosed at 10 AM; EH, 1.0 mg/kg trandolapril dosed at 10 PM; VHCL, vehicle; SHAM, sham-operation. mean ± S.E.M., *P<0.05, †P<0.01 vs the vehicle-treated rats.
0.05 mg/g in the vehicle-treated rats vs. 1.75 ± 0.03 mg/g in the sham-operated rats, P<0.01). A low-dose of trandolapril did not reduce this parameter (2.11 ± 0.05 mg/g in the morning trial, 2.14 ± 0.03 mg/g in the evening trial). A high-dose of trandolapril prevented the development of LV hypertrophy (1.74 ± 0.04 mg/g in the morning trial, 1.92 ± 0.04 mg/g in the evening trial). LV mass in the morning group, which was significantly lower than that in the evening group, was reduced to the control level (sham-operated group) (Fig. 1B).

Serum ACE activity was decreased by the treatment with trandolapril in a dose-dependent manner without a significant difference between the morning and evening trials (Table 1).

The present study showed that the preventive effect of trandolapril against LV hypertrophy was greater when it was given at a resting period (morning) than when it was administered at an active period (evening) in hypertensive rats with aortic banding. A recent study showed a similar finding that a low dose of captopril (15 mg/kg), another ACE inhibitor, dosed at 8 AM comparably regressed the LV hypertrophy compared with its large dose (75 mg/kg) at evening (6 to 8 PM) in rats with two kidney, one-clip (7). Hypertensive LV hypertrophy is an independent risk factor for cardiovascular morbidity and mortality in patients with essential hypertension. Regression of LV mass by antihypertensive drugs may lead to a reduction of cardiovascular events (8). Based on these findings, we hypothesize that the preventive effect of trandolapril against cardiac hypertrophy is greater in hypertensive patients and subsequent improvement of their prognosis are greater during night-time dosing.

Aortic banding at a proximal site to renal arteries induces an activation of renin-angiotensin system (9, 10). Angiotensin II, the primary effector of renin-angiotensin system, is shown to stimulate cardiomyocyte hypertrophy (11). As aortic banding increased blood pressure in the carotid artery, it is likely that hemodynamic overload and the activated renin-angiotensin system cause cardiac hypertrophy in this model.

Duration of the hypotensive effect of ACE inhibitors is greater after dosing at evening than after dosing at morning in hypertensive patients (4, 5). This chronopharmacological event partly depends on a more sustained inhibition of plasma ACE activity during evening treatment (12). However, as blood pressure measured at 22 to 26 h after the final dose of trandolapril did not differ between morning and evening groups in this study, a dosing time-dependent difference in the duration of hypotensive effect might not be involved in the mechanism of the chronopharmacological phenomenon. A previous study had demonstrated that serum renin activity during a resting period is higher than that during an active period in normotensive rats (13). Thus, it remains possible that renin activity in circulating blood also shows a similar daily variation in rats with aortic banding, which might cause a greater fall in blood pressure, especially during a resting period after dosing at morning.

Quinapril, another ACE inhibitor, caused a more sustained inhibition of ACE activity dosed in the evening compared with after dosing in the morning in hypertensive patients (4). In the present study, trandolapril inhibited serum ACE activity in a dose-dependent manner. Contrary to human subjects, no significant difference was observed in this parameter between morning and evening dosings. Therefore, we did not have any data indicating that reduction in plasma angiotensin II concentration, a potent stimulator for cardiac hypertrophy, is greater after dosing of trandolapril at morning. However, it is unclear whether the suppression of angiotensin II production in cardiac tissues depends on the dosing time of trandolapril in hypertensive rats with aortic banding.

In summary, the present study showed that the preventive effect of trandolapril against LV hypertrophy depends on its dosing time in hypertensive rats with aortic banding. Further studies including a continuous blood pressure monitoring with a telemetry and measurement of cardiac angiotensin II concentration are needed to evaluate the mechanism of this interesting event.

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