Inhibitory Effects of a Subdepressor Dose of L-158,809, an Angiotensin II Type 1 Receptor Antagonist, on Cardiac Hypertrophy and Nephropathy Via the Activated Human Renin-Angiotensin System in Double Transgenic Mice With Hypertension

Tatsuya Kai, MD; Keiichi Sugimura, MD; Seijiro Shimada, MD; Atsuhiro Kurooka, MD; Toshihiko Takenaka, MD; Kinji Ishikawa, MD

The effects of L-158,809, an angiotensin II type 1 receptor antagonist, on cardiac hypertrophy and nephropathy were examined using Tsukuba hypertensive mice (THM) carrying both human renin and angiotensinogen genes. Nine male THM aged 20 weeks were assigned to each of a no-dosage group and an L-158,809 dosage group, and L-158,809 was administered for 8 weeks. Nine age-matched male C57BL/6 mice were used as normal control animals. At 28 weeks of age, all of the mice were euthanized. Systolic blood pressure, urinary volume, water intake volume, urinary albumin excretion, heart weight and kidney weight to body weight ratios and a glomerulosclerosis index were measured. In the no-dosage group, the values of all of these parameters were larger than those in the control mice. In the L-158,809 group, all of the parameters showed significant improvement, except for blood pressure, which was not significantly different from that in the no-dosage group. These results suggest that the renin-angiotensin system played a crucial role in the cardiac hypertrophy and nephropathy in THM, and that L-158,809 exhibited strong curative effects on cardiac hypertrophy and nephropathy by blocking the angiotensin II type 1 receptor. (Jpn Circ J 1998; 62: 599–603)

Key Words: Angiotensin II; Cardiac hypertrophy; Nephropathy; Renin-angiotensin system; Transgenic mice

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The Tsukuba hypertensive mouse (THM) is a hypertensive animal model prepared by introducing human renin and angiotensinogen genes into C57BL/6 mouse! In THM, the cause of hypertension is known to be a single factor, an enhancement of the renin-angiotensin system (RAS). In THM, angiotensin II (AII) is 3–5 times higher in serum and that in the heart and kidney is 4–5 times higher compared to C57BL/6 mice.2 At 6 weeks of age, when blood pressure measurement becomes possible, THM already show elevated blood pressure. In THM, the occurrence of severe cardiac hypertrophy and glomerulosclerosis, in comparison with the degree of hypertension, has been reported3.

L-158,809 [5,7-dimethyl-2-ethyl-3-[2’-(1H-tetrazol-5-yl)[1,1’]-biphenyl-4-yl]-methyl]-3H-imidazo [4,5-b] pyridine] is a selective AII receptor antagonist with high potency, good per os absorption, long duration and antihypertensive efficacy after a single dose equal to that of angiotensin converting enzyme inhibition, and does not have AII agonist activity.4,5 The affinity of L-158,809 for the AII type 1 receptor (AT1R) and its potency for the inhibition of AII responses in a variety of tissues and in vitro preparations is subnanomolar (IC50=0.2–0.8 nmol/L). L-158,809 is 50–200 times more potent than losartan, an AT1R antagonist, and 5–15 times more potent than the metabolite, EXP3174. Indeed, the affinity of L-158,809 for AT1R is similar to that of the natural hormone, AII. With respect to the subtypes of AII receptors, L-158,809 has high selectivity for the AT1R, as does losartan, unlike PD-123,177 and PD-121,981, which are selective for AII type 2 receptor (AT2R). Unlike other AT1R antagonists, the inhibitory activity of L-158,809 is observed shortly after per os administration, and the duration of activity is greater than 24 h for both intravenous and per os administration. Moreover, the per os to intravenous potency ratio is 0.8. Therefore, L-158,809 is the most potent AT1R antagonist described to date.

In the present study, L-158,809 was administered to THM, and its curative effects on cardiac hypertrophy and nephropathy were examined.

Methods

Animals and Drugs

Nine male THM were assigned to each of an L-158,809 dosage group (0.3 mg/kg per day) and a no-dosage group. Nine age-matched male C57BL/6 mice were used as normal controls. In the L-158,809 group, the drug was dissolved in drinking water and administered for 8 weeks from the age of 20 weeks. All the mice were euthanized at 28 weeks of age. The mice were bred in an air-conditioned room at 22±2°C and humidity of 50±10%, with a 12-h light period (from 7.00 h to 19.00 h). They were fed a breeding...
Injection of pentobarbital sodium (Pitman Moore Inc, Mundelein, PA, USA) at a dose of 0.75 mg/g body weight and then weighed. The abdomen was incised and the heart and kidneys were extracted. The heart was weighed after removal of the atria, and the heart to body weight ratio was calculated. The mean weight of the left and right kidneys was used in the calculation of the kidney weight to body weight ratio.

**Measurement of the Glomerulosclerosis Index**

The kidneys extracted at the time of euthanasia were fixed in a 10% formalin solution (Wako Pure Chemicals, Osaka, Japan), embedded in paraffin, sectioned at 3 μm thickness with a microtome, and subjected to hematoxylin and eosin (HE) stain, periodic acid Schiff (PAS) stain and Masson–trichrome stain. On each section, 200 randomly selected glomeruli were examined, and the glomerulosclerosis index was calculated according to the method of Kohara et al. Discrimination between sclerosis and hyaline glomeruli was stained red by PAS staining and blue or green by Masson–trichrome staining, and hyaline glomeruli were stained red by both PAS staining and Masson–trichrome staining.

**Statistical Analysis**

One-way analysis of variance was used in the statistical analysis. The data obtained are expressed as the mean ± standard deviation. Probability values less than 0.01 were considered significant.

**Results**

The results are summarized in Table 1. The systolic blood pressure in the no-dosage group was significantly higher than that in the control group, by about 40 mmHg. In the L-158,809 group, the pressure was not significantly different from that in the no-dosage group. The urinary volume and water intake volume in the no-dosage group were significantly higher than those in the control group, while the volumes in the L-158,809 group were significantly less than those in the no-dosage group. The amount of urinary albumin excretion in the no-dosage group was about 14 times that in the control group, whereas that in the L-158,809 group was significantly less than that in the no-dosage group. Both the heart weight and kidney weight to body weight ratios in the no-dosage group were significantly lower than the ratios in the no-dosage group. The glomerulosclerosis

**Table 1 Results of the Measurements**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C57BL/6 mice</th>
<th>No-dosage group</th>
<th>L-158,809 group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>93±2*</td>
<td>135±4†</td>
<td>133±4</td>
</tr>
<tr>
<td>Urinary volume (ml/day)</td>
<td>1.4±0.3**</td>
<td>7.2±3.6†</td>
<td>4.2±2.1*</td>
</tr>
<tr>
<td>Water intake volume (ml/day)</td>
<td>4.4±1.2**</td>
<td>11.5±4.5†</td>
<td>8.2±2.3*</td>
</tr>
<tr>
<td>Urinary albumin excretion (mg/day per 100g body weight)</td>
<td>17±3**</td>
<td>246±13†</td>
<td>147±15*</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>43±4**</td>
<td>30±5†</td>
<td>35±4*</td>
</tr>
<tr>
<td>Heart weight (g)</td>
<td>0.14±0.01**</td>
<td>2.1±0.07†</td>
<td>0.18±0.03*</td>
</tr>
<tr>
<td>Mean kidney weight (g)</td>
<td>0.23±0.02</td>
<td>0.21±0.02</td>
<td>0.21±0.03</td>
</tr>
<tr>
<td>Heart to body weight ratio (%)</td>
<td>0.3±0.02**</td>
<td>0.72±0.25†</td>
<td>0.52±0.10*</td>
</tr>
<tr>
<td>Kidney to body weight ratio (%)</td>
<td>1.0±0.09†</td>
<td>1.4±0.18†</td>
<td>1.16±0.08*</td>
</tr>
<tr>
<td>Glomerulosclerosis index (%)</td>
<td>0.4±0.7†</td>
<td>11.0±2.8†</td>
<td>5.5±1.3*</td>
</tr>
</tbody>
</table>

*p<0.01 compared with no-dosage group; †p<0.01 compared with L-158,809 group.

Measurement of Systolic Blood Pressure

Systolic blood pressure was measured with an automatic mouse blood pressure recorder (Model BP-98A, Softron Co, Tokyo, Japan) by the tail-pulse pick-up method without anesthesia, around noon.

Measurement of Urinary Volume, Water Intake Volume and Urinary Albumin Excretion

The urinary volume and water intake for 24 h were measured using metabolic cages (CL-0305, Nihon Clea Co, Tokyo, Japan). The amount of urinary albumin excretion was measured by immunonephelometry using a TIA micro alb kit (Nitto-boseki Co, Tokyo, Japan).

Measurement of Heart Weight and Kidney Weight To Body Weight Ratios

Each mouse was anesthetized by an intraperitoneal injection of pentobarbital sodium (Pitman Moore Inc, Mundelein, PA, USA) at a dose of 0.75 mg/g body weight and then weighed. The abdomen was incised and the heart and kidneys were extracted. The heart was weighed after removal of the atria, and the heart to body weight ratio was calculated. The mean weight of the left and right kidneys was used in the calculation of the kidney weight to body weight ratio.
index in the no-dosage group was significantly higher than that in the control group, and the index in the L-158,809 group was significantly lower than that in the no-dosage group. In the histological examination of the kidneys, marked infiltrations of mainly plasma cells around the arterioles and vascular fibroid necrosis were found in the no-dosage group (Fig 1), whereas no such changes were found in the control or L-158,809 groups.

**Discussion**

Various types of hypotensive drugs are available and a decrease in blood pressure can be achieved easily. However, prevention or amelioration of cardiac hypertrophy and other organ failure, as well as the normalization of blood pressure, are necessary for the comprehensive treatment of hypertension. Many studies have been done on the effectiveness of various depressors in controlling hypertensive organ failure. Among them, angiotensin converting enzyme inhibitor (ACEI) was shown to be the most effective drug for improving organ disorder when used in hypertensive cardiac hypertrophy\(^1\) and hypertensive nephropathy.\(^5\)\(^,\)\(^10\)

L-158,809 is a potent competitive and specific antagonist of AT1R.\(^1\) Unlike ACEI, AT1R antagonists have no ability to inhibit the degradation of kinin, and therefore produce less side effects such as coughing and vascular edema; in addition, they have the advantage of blocking the role of the RAS by administering lisinopril to THM at a dose that did not cause significantly controlled. Based on this finding, we have reached the conclusion that the RAS is closely associated with the occurrence of cardiac hypertrophy and renal disorders in THM. However, some researchers suggest that the organ protection of ACEIs is due to the inhibition of AII\(^15\)\(^–\)\(^20\) while others suggest that it is due to an enhancement of bradykinin rather than inhibition of AII.\(^21\)\(^–\)\(^25\) Moreover, it is reported that an ACEI increased the \([\mathbf{1}]\) receptor density of myocytes and augmented the response to isoproterenol, while an AT1R antagonist did not have the same effects.\(^26\) In addition, captopril, one of the ACEI, has the cardiac protective effect of increasing the tissue levels of ATP, total adenine nucleotides, energy charge potential and creatine phosphate; these effects are independent of the blunting of AII formation.\(^27\)

In most of the studies in which AT1R antagonists were administered, it has been suggested that AT1R antagonists had an organ protective action.\(^6\)\(^,\)\(^13\)\(^–\)\(^16\),\(^28\) However, Okuda et al reported that losartan controlled cardiac hypertrophy at a dose that caused significant hypotension, but did not control cardiac hypertrophy at a dose that did not cause hypotension, and they concluded that AT1R antagonists alleviated cardiac hypertrophy because of their hypertensive action.\(^29\) In the present study, we administered L-158,809, an AT1R antagonist, to THM at a dose that did not cause hypotension, to determine whether it would prevent cardiac hypertrophy and renal disorders in a manner similar to that of lisinopril. As a result, the proteinuria, glomerulosclerosis, renal hypertrophy and cardiac hypertrophy of the L-158,809 group improved significantly, although their blood pressure remained the same. Mizuno et al reported that losartan reduced left ventricular hypertrophy and tissue AII contents in rats without lowering the serum AII, and they concluded that the locally generated cardiac, but not circulating, RAS may be important for cardiac hypertrophy.\(^30\) It has been also reported that the tissue AII level is reduced by the administration of ACEI.\(^11\)\(^,\)\(^12\) Because the tissue AII concentration in THM is about 4–5 times higher than in C57BL/6 mice; we suggest that the cardiac hypertrophy and nephropathy in THM were induced by enhanced tissue RAS, and that L-158,809 improved the tissue RAS.

In the present study, the administration of L-158,809 significantly controlled cardiac hypertrophy and renal disorders, but no significant change in systolic blood pressure was seen. The systolic blood pressure was measured around noon. Mice usually drink water during the night up until early in the morning, and they may have hypotension and organ protection during the hours when the blood pressure is not measured. However, the hypotensive action of the per os administration of L-158,809 lasted for 24 h or longer, and it did not completely disappear even 48 h after administration;\(^5\) so it was less likely that the mice in the present study had significant hypotension during certain hours. At the same time, it is necessary to consider the possibility that organ protection comes not from AT1R inhibition but from a nonspecific action of L-158,809 itself.

It is also necessary to determine whether the alleviation of the cardiac hypertrophy was produced by inhibition of either the hypertrophy of myocardial cells or interstitial fibrosis. AII is reported to accelerate the hypertrophy of myocardial cells as well as interstitial fibrosis.\(^33\)\(^–\)\(^37\) Aldosterone, the secretion of which is stimulated by AII, is also reported to directly accelerate the hypertrophy of myocardial cells as well as interstitial fibrosis.\(^38\) Inasmuch as AII has a stimulating effect on aldosterone secretion via AT1R,\(^39\) the alleviation of cardiac hypertrophy is thought to be the result of inhibiting both the hypertrophy of myocardial cells and interstitial fibrosis.

It is known that mechanical stresses such as high blood pressure accelerate the hypertrophy of cardiac muscles and renal glomerulosclerosis.\(^30\)\(^–\)\(^42\) AII has been reported to produce the same effects.\(^43\)–\(^46\) This action is thought to be mediated mainly by AT1R.\(^11\),\(^47\),\(^48\) In contrast, the inhibition of cell proliferation and protein synthesis, as actions mediated via AT2R, was reported.\(^48\)–\(^50\) From these results, it appears that L-158,809 exhibits a marked improving effect on cardiac hypertrophy and nephropathy by blocking the effects via AT1R. In addition, there is a possibility that the administration of L-158,809 enhanced the effects via AT2R as well as blocking the effects via AT1R.\(^51\)

Histological examination of the kidney, revealed distinct infiltration of inflammatory cells around the arterioles and vascular fibroid necrosis in the no-dosage group, while these were not observed in either the L-158,809 or control groups. The infiltration of inflammatory cells to the region surrounding arterioles also occurs in other animal models with chronic hypertension, such as spontaneously hyper-
tensive rats, salt-sensitive Dahl rats and DOCA-salt hypertensive rats, and it is known that an immunological abnormality is involved in this occurrence. Renal disorders can occur by an immunological mechanism or by a nonimmunological mechanism, such as abnormal renal hemodynamics, and activation of the RAS is thought to play a major role in the latter mechanism. In the present study, the infiltration of inflammatory cells to the region surrounding arterioles was seen in the no-dosage group with accelerated RAS, while such changes were not seen in the L-158,809 group, whose AII was inhibited without causing hypotension. These findings suggest the possibility that the acceleration of AII is in part associated with an immunological mechanism, such as abnormal renal hemodynamic effect of chronic versus acute lisinopril administration in the rabbit. 

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Effects of Angiotensin II Receptor Antagonist

and carotid wall thickening. Circulation 1994; 89: 952 – 954