Cardiovascular Effects of L-158,809, a New Angiotensin Type 1 Receptor Antagonist, Assessed Using the Halothane-Anesthetized In Vivo Canine Model

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ABSTRACT—L-158,809 is a new angiotensin II type 1 receptor antagonist. We simultaneously assessed its antagonistic potency and cardiovascular effects with the halothane-anesthetized in vivo canine model (n = 5). L-158,809 was intravenously infused over 10 min at escalating doses of 0.03, 0.3 and 3 mg/kg. Angiotensin II (0.1 μg/kg, i.v.)-induced vasopressor and negative inotropic responses were significantly suppressed from the low dose L-158,809. Meanwhile, L-158,809 did not affect any of the cardiovascular parameters except that QTc was slightly shortened after the high dose administration. These results support the previous in vitro knowledge that L-158,809 is a highly selective angiotensin II receptor antagonist.

Keywords: L-158,809, Angiotensin II, QT interval

L-158,809 is a highly selective angiotensin II type 1 (AT₁) receptor antagonist whose affinity for AT₁ receptors has been shown to be greater than the existing angiotensin II receptor antagonists (1). Indeed, the affinity of L-158,809 for AT₁ receptors is similar to that of angiotensin II itself (1). Moreover, a recent experimental study (2) has shown that L-158,809 attenuates early left ventricular remodeling during canine myocardial infarction. However, information is still limited regarding the relationship between the doses of L-158,809 that block angiotensin II receptor and those that affect the cardiovascular variables. In this study, we simultaneously assessed the in vivo angiotensin II receptor blocking action and cardiovascular effects of L-158,809, using the halothane-anesthetized, closed-chest canine model (3 – 5).

All experiments were performed according to Guidelines for Animal Experiments, Yamanashi Medical University. Experiments were carried out using beagle dogs weighing approximately 10 kg. The animals had been fed with about 240 g/day of standard dog foods (CD-55α; CLEA Japan, Tokyo) containing 0.41 g/100 g of NaCl for >1 month in the Animal Laboratory for Research of Yamanashi Medical University. Dogs were anesthetized initially with thiopental sodium (30 mg/kg, i.v.). After intubation, 1.0% halothane vaporized with 100% oxygen was inhaled with a ventilator (SN-480-3; Shinano, Tokyo). Tidal volume and respiratory rate were set at 20 ml/kg and 15 strokes/min, respectively. To prevent blood clotting, heparin calcium (100 IU/kg) was intravenously administered.

The surface lead II ECG was obtained from the limb electrodes. Corrected QT interval (QTc) was obtained using Bazett’s formula (6). The systemic blood pressure was measured at the left femoral artery. A thermodilution catheter (TC-704; Nihon Kohden, Tokyo) was positioned at the right side of the heart through the right femoral vein. The cardiac output was measured by a standard thermodilution method using a cardiac output computer (MFC-1100, Nihon Kohden). The total peripheral vascular resistance (TPR) was calculated using the following basic equation: TPR = mean blood pressure / cardiac output. A quad-polar electrodes catheter was positioned at the non-coronary cusp of the aortic valves through the right femoral artery to obtain the His bundle electrogram. A pig-tail catheter was positioned at the left ventricle through the left femoral artery to measure the left ventricular pressure. The maximum upstroke velocity of the left ventricular pressure (LVdP/dt max) and the left ventricular end-diastolic pressure (LVEDP) were obtained during the sinus rhythm to estimate the contractility and the preload to the left ventricle, respectively.

A bi-directional steerable monophasic action potential (MAP) recording/pacing combination catheter (1675P; EP Technologies, Inc., Sunnyvale, CA, USA) was positioned...
at the endocardium of the interventricular septum in the right ventricle through the left femoral vein to obtain MAP signals. The signals were amplified with a DC preamplifier (Model 300, EP Technologies Inc.). The duration of the MAP signals was measured as an interval, along a line horizontal to the diastolic baseline, from the MAP upstroke to the desired repolarization level. The interval (ms) at 90% repolarization was defined as MAP\(_{90}\).

The heart was electrically driven using a cardiac stimulator (SEC-3102, Nihon Kohden) with the pacing electrodes of the MAP recording/pacing combination catheter placed in the right ventricle. The stimulation pulses were rectangular in shape, 1 – 2 V of amplitude (about twice the threshold voltage) and 1 ms in duration. The MAP\(_{90}\) was measured during sinus rhythm (MAP\(_{90}(\text{sinus})\)) and at a pacing cycle length of 400 ms (MAP\(_{90}(\text{CL400})\)) and 300 ms (MAP\(_{90}(\text{CL300})\)). The effective refractory period (ERP) of the right ventricle was assessed by the programmed electrical stimulation. The pacing protocol consisted of 5 beats of basal stimuli in a cycle length of 400 ms followed by an extra-stimulus of various coupling intervals. Starting in late diastole, the coupling interval was shortened in 5 – 10-ms decrements until refractoriness occurred. The final repolarization phase of the ventricle, namely, the relative refractory period, was estimated by the difference between the ERP and MAP\(_{90(\text{CL400})}\) at the same site. In this study, post-repolarization refractoriness, PRR = ERP – MAP\(_{90(\text{CL400})}\), was calculated to estimate the electrical vulnerability of the ventricular muscle, as described before (3 – 5).

The systemic blood pressure, left ventricular pressure, ECG, His bundle electrogram and MAP signals were monitored with a polygraph system (RM-6000, Nihon Kohden), and analyzed using a real time full automatic data analysis system (MP/VAS 3 for Macintosh, ver 1.0; Physio-Tech, Tokyo). Each measurement of ECG, MAP, and atrio-His (AH) and His-ventricular (HV) intervals was the mean of three recordings of consecutive complexes. The cardiovascular variables were assessed in the following order: The cardiac output was measured three times. The ECG, His bundle electrogram, systemic and left ventricular pressure, and MAP signal were recorded under sinus rhythm. Next, MAP signals were recorded during the ventricular pacing at the cycle lengths of 400 and 300 ms. Finally, ERP was measured at the same site where MAP was recorded. All parameters described above were usually obtained within 1 min at each recording time point.

After each basal control cardiovascular value was assessed, 0.1 μg/kg of angiotensin II was intravenously administered to estimate the antagonistic action of L-158,809. Next, 0.3 mg/kg of L-158,809 was additionally administered over 10 min and each parameter was observed in the same manner. Then, 0.1 μg/kg of angiotensin II was intravenously injected. Finally, 3.0 mg/kg of L-158,809 was additionally administered over 10 min and each parameter was observed at 5, 10, 15, 20, 30, 45 and 60 min after the start of the infusion. After each assessment at 30 and 60 min was over, 0.1 μg/kg of angiotensin II was intravenously injected. The cardiovascular effects of the respective solvents for each dose (0.15, 1.5 and 15% of saturated NaHCO\(_3\) solution) and their effects on the angiotensin II-induced responses were similarly assessed.

L-158,809; 2-ethyl-5,7-dimethyl-3-[([2’-(1H-tetrazol-5-yl)]1,1’-biphenyl)-4-yl]methyl]-3H-imidazo[4,5-B]pyridine (MW = 409,498) was generously provided by Merck (Whitehouse Station, NJ, USA). Forty milligrams of L-158,809 was dissolved in 3 ml of saturated NaHCO\(_3\) solution and diluted with saline to a total volume of 20 ml, which gave and L-158,809 solution of 2 mg/ml. This solution was further diluted with saline to 0.2 and 0.02 mg/ml. L-158,809 solutions in concentrations of 0.02, 0.2 and 2.0 mg/ml were used for preparing the doses of 0.03, 0.3 and 3.0 mg/kg, respectively. The following drugs were purchased: angiotensin II (Peptide Institute, Inc., Osaka), thiopental sodium (Tanabe, Osaka), halothane (Takeda, Tokyo) and heparin calcium (Mitsui, Tokyo). Angiotensin II was dissolved in saline in a concentration of 1 μg/ml.

Data are presented as the mean ± S.E.M. The statistical significance within a parameter was evaluated by the paired t-test or one-way, repeated-measures analysis of variance (ANOVA) followed by Contrasts for mean values comparison. A P value <0.05 was considered significant.

Typical tracings of angiotensin II-induced responses in the heart rate, blood pressure and LVdp/dt are depicted in Fig. 1A, and the blocking action of L-158,809 for angiotensin II-induced cardiovascular responses are summarized in Fig. 1B (n = 5). Before the L-158,809 treatment, angiotensin II-induced change was +26 ± 5 mmHg in the mean blood pressure and −246 ± 45 mmHg/s in the LVdp/dt\(_{\text{max}}\), whereas the heart rate tended to decrease, which did not achieve statistical significance. Administration of angiotensin II did not induce any arrhythmias. Infusion of L-158,809 significantly suppressed these angiotensin II-induced vasopressor and negative inotropic responses in a dose-related manner. Meanwhile, infusion of vehicle NaHCO\(_3\) solution did not affect the angiotensin II-induced cardiovascular responses (n = 5, not shown in the figure), indicating the absence of tachyphylaxis in the angiotensin II-induced responses in the current protocol.

The time courses of the heart rate, mean blood pressure and TPR; those of the cardiac output, LVdp/dt\(_{\text{max}}\) and
LVEDP; those of the electrophysiological parameters during the sinus rhythm; and those during the ventricular pacing after the L-158,809 infusion are summarized in Fig. 2 A, B, C and D, respectively. No significant change was induced by the L-158,809 infusion in any variable except that QTc decreased at 30–60 min after the high-dose treatment. In addition, TPR tended to be decreased after the infusion of L-158,809, which did not achieve statistical significance. The administration of vehicle solution did not affect any of the cardiovascular parameters.

Given the limited information on L-158,809 regarding the relationship between the angiotensin II receptor blocking potency and cardiovascular effects in vivo, we assessed it using the well-established, halothane-anesthetized canine model (3–5). The dosage of L-158,809 was determined based on the previous reports (7), in which anti-hypertensive action was observed with the use of 0.1–0.3 mg/kg of L-158,809 against renin-dependent hypertensive rats and volume-depleted rhesus monkeys. As clearly shown in the results, L-158,809 potently suppressed the angiotensin II-induced vasopressor response, with a potency similar to those reported previously (7). We also confirmed the angiotensin II-induced negative inotropic effect in this study, although the substance has been reported to exert positive (8), no (9), negative (10), or biphasic inotropic effects (11). L-158,809 attenuated the angiotensin II-induced negative inotropic response in a dose-related manner, which has not been reported elsewhere. More importantly, L-158,809 did not affect any of the cardiovascular parameters except for QTc.

Since typical AT1 receptor antagonists like candesartan and losartan have been reported to modify the delayed rectifier potassium currents at therapeutic concentrations leading to the prolongation of action potential duration in
Fig. 2. Summary of the cardiovascular profile of L-158,809 (n = 5). A: Time courses of heart rate, mean blood pressure (mBP) and total peripheral vascular resistance (TPR). Their pre-drug control values were 114 ± 8 beats/min, 108 ± 7 mmHg and 81 ± 9 mmHg · min/L, respectively. B: Time courses of cardiac output, maximum upstroke velocity of left ventricular pressure (LVdP/dtmax) and left ventricular end-diastolic pressure (LVEDP). Their pre-drug control values were 1.39 ± 0.09 l/min, 2139 ± 123 mmHg/s and 11 ± 1 mmHg, respectively. C: Time courses of QTc (circles) and QT interval (squares), PR interval (circles) and QRS width (triangles), MAP90(sinus) (squares), AH (circles) and HV intervals (triangles). Their pre-drug control values were 356 ± 7 ms, 261 ± 13 ms, 94 ± 4 ms, 62 ± 3 ms, 254 ± 10 ms, 67 ± 4 ms and 28 ± 2 ms, respectively. D: Time courses of MAP90(CL300), MAP90(CL400), ERP and PRR. Their pre-drug control values were 218 ± 7, 235 ± 8, 209 ± 3 and -26 ± 8 ms, respectively. MAP90 represents the duration of the monophasic action potential at 90% repolarization level. MAP90(CL300) and MAP90(CL400) during the sinus rhythm; MAP90(CL300) and MAP90(CL400) during the ventricular pacing at the cycle lengths of 300 and 400 ms, respectively; AH, atrio-His; HV, His-ventricular ERP, effective refractory period; and PRR, postrepolarization refractoriness. Data are presented as the mean ± S.E.M. The closed symbols represent the significant differences from each control value (C) at *P*<0.05.
vitro (12, 13), we precisely assessed the electrophysiological effects of L-158,809 under the monitoring of MAP and His bundle electrogram. As shown in the results, the drug did not affect any of the electrophysiological parameters in this study except that QTc was shortened after the high-dose infusion. It should be noted that MAP was hardly affected during the whole experimental period, but the heart rate tended to decrease after the high dose infusion. These observations may suggest that QT interval is overcorrected by Bazett’s formula at the heart rate of >100 beats/min (5) and/or that an extremely high dose of L-158,809 might promote the repolarization process via nonspecific mechanisms during the sinus rhythm.

In summary, the present results support the previous in vitro knowledge that L-158,809 is a highly selective angiotensin II receptor antagonist, and they suggest that constitutional angiotensin II may not be important in regulating the canine cardiovascular system under physiologically maintained mechanical and electrical conditions. In addition, the data shown in this study will provide convenient guidelines for comparing the angiotensin II receptor blocking potency and potential cardiovascular adverse effects of new angiotensin II receptor antagonists.

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