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Antihypertensive Effects of Enantiomers of Amlodipine Camsylate, a Novel Salt of Amlodipine

Kwang-Seok OH[1], Maeng Sup KIM[2], and Byung Ho LEE[1]*

[1]Center for drug discovery technology, Korea Research Institute of Chemical Technology, Yuseong, Daejon, 305-343, Korea

[2]Central Research Institute, Hanmi Pharm Co., Ltd., Sungnam, 463-400, Korea

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Abstract – The vascular relaxant effects on isolated rat aorta of amlodipine camsylates (S-, R-enantiomer, and R/S-racemate), were evaluated and compared with that of S-amlodipine besylate. Furthermore, antihypertensive effects were measured in spontaneously hypertensive rat (SHR). The S-amlodipine camsylate concentration-dependently inhibited Ca^{2+}-induced contraction of rat aorta with a very slow onset of action (reached its maximum at 3.5h; ED_{50}: 1.50 ± 0.24 nM), having a potency 2-fold higher than those of R/S-amlodipine camsylate (ED_{50}: 3.36 ± 0.91 nM) and similar to those of S-amlodipine besylate (ED_{50}: 1.44 ± 0.14 nM), whereas the R-amlodipine camsylate has 590-fold lower vasorelaxant activity (ED_{50}: 886.4 ± 49.7 nM). In SHR, orally administered S-amlodipine camsylate produced a dose-dependent and long-lasting (>10 h) antihypertensive effect (ED_{20}: 0.89 mg/kg), with a potency 2-fold higher than those of R/S-amlodipine camsylate (ED_{20}: 1.82 mg/kg) and similar to those of S-amlodipine besylate (ED_{20}: 0.71 mg/kg). In contrast, the R-amlodipine camsylate has no effect even-though administrated high concentration 10 mg/kg. These results suggest that S-amlodipine camsylate has the potency and long-lasting antihypertensive activity as single enantiomer drug, and its antihypertensive effect is not significantly different to that of S-amlodipine besylate.

Key words □ antihypertension, amlodipine, camsylate, calcium antagonist, spontaneously hypertensive rat

INTRODUCTION

Calcium channel blockers are clinically useful vasodilators, used widely in the treatment of hypertension and ischemic heart disease. Among several calcium channel blockers, amlodipine has popular prescribed in the management of angina and hypertension, because its pharmacokinetic profile differs from those of other dihydropyridine calcium antagonists (Abernethy, 1989; Meredith and Elliott, 1992). Amlodipine as a third-generation dihydropyridine calcium antagonist, has lower hepatic extraction ratio and, consequently, higher bioavailability (Beresford *et al.*, 1988; Walker *et al.*, 1994). However, amlodipine is composed of racemic mixture (Tucker, 2000; Burke and Henderson, 2002). Essentially, the calcium channel-blocking effect is confined to the S-amlodipine (Arrowsmith *et al.*, 1986; Goldmann *et al.*, 1992), whereas the R-amlodipine, even-though causes nitric oxide-mediated vasodilation, has 1000-fold lower calcium channel blocking activity (Zhang *et al.*, 2002). In view of the antihypertensive and antianginal effects, R-amlodipine might be considered to be inactive compound (Rentsch, 2002). Therefore, the successful development of an amlodipine formulation composed wholly of S-amlodipine through chiral switching, might have been anticipated. Moreover, research interest has focused on the issue of the new S-amlodipine formulation to improve pharmacokinetic profile. Recently, amlodipine camsylates such as R-, S- enantiomer and R/S-racemate were developed by Hanmi Pharm. Co., as a generic salt form of amlodipine besylate, commercially available form. The amlodipine camsylate was reported to improve photochemical stability compared to amlodipine besylate (Park *et al.*, 2004, 2006).

The present study was designed to evaluate the vasorelaxant effect of amlodipine camsylates such as S-, R-enantiomer, and R/S-racemate on isolated rat aorta and antihypertensive effects in spontaneously hypertensive rats (SHRs), which were compared with those of S-amlodipine besylate as reference formulation.

*Corresponding author
Tel: +82-42-860-7415, Fax: +82-42-861-4246
E-mail: bhlee@krict.re.kr

MATERIALS AND METHODS

Animals

Male SHRs (13-14 weeks) and male Sprague-Dawley (SD) rats (380-420g) were purchased from Charles River (Tokyo, Japan) and Orient Co. (Seoul, Korea), respectively. The animals were conditioned for 1 week at 22.5 ± 1°C with a constant humidity of 55 ± 5%, a cycle of 12-h light/dark, and free access to food and tap water.

Materials

The amlodipine camsylates (S-, R-enantiomers and R/S-racemate) and S-amlodipine besylate (Fig. 1) were supplied by Hanmi Pharm. Co. (Seoul, Korea), which were prepared according to procedures previously described (Lee *et al.*, 2006; Spargo, 1995) and dissolved in distilled water. The purities of all compounds were above 99.0%. Reagents for the Krebs-Henseleit buffer used in the isolated aorta experiment were purchased from Junsei (Tokyo, Japan). All drugs and reagents were prepared just prior to use.

Vasorelaxant effects on isolated rat aorta

Thoracic aorta was isolated from male SD rat and each aorta was cut into 2-3 mm wide rings with extreme care to preserve the endothelium (Burges *et al.*, 1987; Shin *et al.*, 1998; Lee *et al.*, 2001). The aortic preparations were suspended between wire hooks in an organ bath containing 20 ml of Ca^{2+}-free Krebs-Henseleit buffer (mM: NaCl, 118.0; KCl, 45; $CaCl_2$, 2.5; $NaHCO_3$, 25; $MgSO_4$, 1.2; KH_2PO_4, 1.2; and glucose, 11.0) bubbled with a gas mixture (95% O2, 5% CO2) and maintained at 37°C. The aortic preparations were allowed to equilibrate for 60 min under 2 g of resting tension. Isometric contraction was measured with a force displacement transducer (Grass FT03, Grass Ins., Quincy, MA, USA) and displayed on a chart recorder (Multicorder MC 6625, Hugo Sachs Electronic, Hugstetten-March, Germany). The rates of onset of R/S-, S- and R-amlodipine camsylates and S-amlodipine besylate were determined as follows: contractions to 2 mM $CaCl_2$ were evoked at 30 min intervals, each cycle comprising 15 min exposure to Ca^{2+} followed by washout with fresh Ca^{2+}-free Krebs-Henseleit buffer and 15 min recovery. Following an initial conditioning response, which was disregarded, the next two responses served as controls and were averaged, the tissues were then washed again with fresh Ca^{2+}-free Krebs-Henseleit buffer containing either R/S-amlodipine camsylate (1, 3 or 10 nM), S-amlodipine camsylate (0.5, 1.5, or 5 nM), R-amlodipine camsylate (300, 1000, or 3000 nM), S-amlodipine besylate (0.5, 1.5, or 5 nM), or vehicle (distilled water). Further Ca^{2+} responses were then obtained as before, using the drug-containing Ca^{2+}-free Krebs-Henseleit buffer for all subsequent washout steps, such that drug exposure times varied from 0.5 and 3.5 h. Results were expressed as a percent of control contractile force before administrating the drugs.

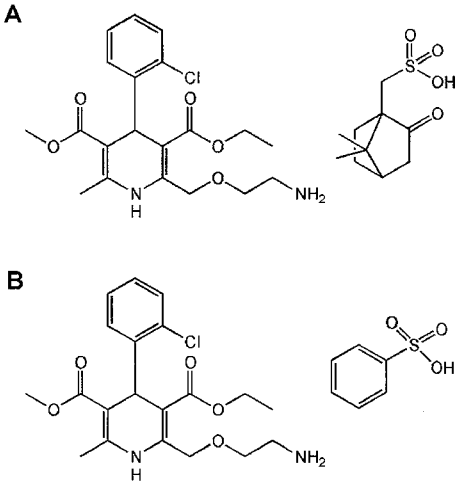

Fig. 1. Chemical structures of amlodipine camsylate (A) and amlodipine besylate (B).

Antihypertensive effects in SHR

The measurements of systolic blood pressure and heart rate were made by the tail-cuff method (Yamanaka *et al.*, 1991; Lee *et al.*, 1998, 1999). Rat tails were occluded with an appropriate size tubular tail cuff (7/16 inch, 12 mm) connected to photoplethysmograph (Model 31, IITC Life Sci., Woodland Hills, CA, USA) and pulse were detected as the cuff pressure was lowered. To measure the blood pressure, rats were prewarmed at 32 °C for 5-10 min in a restraining cage in a warming box. Rats were allowed to habituate to this procedure during at least 2 weeks before experiments. SHRs were randomly divided into five groups. SHRs with systolic blood pressure of more than 170 mmHg were used in this study. All rats were treated with oral administration of either R/S-amlodipine camsylate (1, 3 or 10 mg/kg), S-amlodipine camsylate (0.5, 1.5 or 5 mg/kg), R-amlodipine camsylate (10 mg/kg), S-amlodipine besylate (0.5, 1.5 or 5 mg/kg), or vehicle (distilled water). Results were expressed as percentage changes from baseline systolic blood pressure and heart rate.

Statistical analysis

All values are expressed as mean ± S.E.M. Data were analyzed by unpaired *t*-test and one-way analysis of variance (ANOVA) followed by the Dunnett's test for multiple comparisons (Sigma Stat, Jandel Co., San Rafael, CA, U.S.A.). In all comparisons, the difference was considered to be statistically significant at $p < 0.05$.

RESULTS

Vasorelaxant effects on isolated rat aorta

To evaluate the peripheral vasodilating activity of R/S-, S- and R-amlodipine camsylates, those effects on Ca^{2+}-induced aortic constriction were measured and compared with that of S-amlodipine besylate (Fig. 2, 3). The R/S-, S-amlodipine camsylate and S-amlodipine besylate concentration-dependently inhibited Ca^{2+}-induced aortic contraction with very slow action, the peak effect being reached 3.5h at all concentrations used in this study. The maximal Ca^{2+}-induced aortic constriction of S-amlodipine camsylate (83.8 ± 7.0, 48.7 ± 6.1 and 14.3 ± 1.7% at concentration of 0.5, 1.5 and 5 nM, respectively) were not significantly different with those of S-amlodipine besylate (79.7 ± 4.1, 46.7 ± 2.3 and 16.2 ± 2.5% at concentration of 0.5, 1.5 and 5 nM, respectively). In case of R/S-amlodipine camsylate, the maximal Ca^{2+}-induced aortic constriction was 76.6 ± 5.6, 49.7 ± 4.9 and 16.4 ± 5.0% at concentration of 1, 3 and 10 nM, respectively. The ED_{50} value (i.e. the dose that inhibited contractile response to Ca^{2+} by 50%) of S-amlodipine camsylate (1.50 ± 0.24 nM) were 2-fold higher than that of R/S-amlodipine camsylate (3.36 ± 0.91 nM) and similar with that of S-amlodipine besylate (1.44 ± 0.14 nM). In contrast, the maximal Ca^{2+}-induced aortic constriction of R-amlodipine camsylate were 88.8 ± 2.6% at concentration of 300 nM, 46.0 ± 4.1% at concentration of 1,000 nM, and 4.9 ± 1.1% at concentration of 3,000 nM, respectively (Fig. 2) and ED_{50} value was 886.4 ± 49.7 nM.

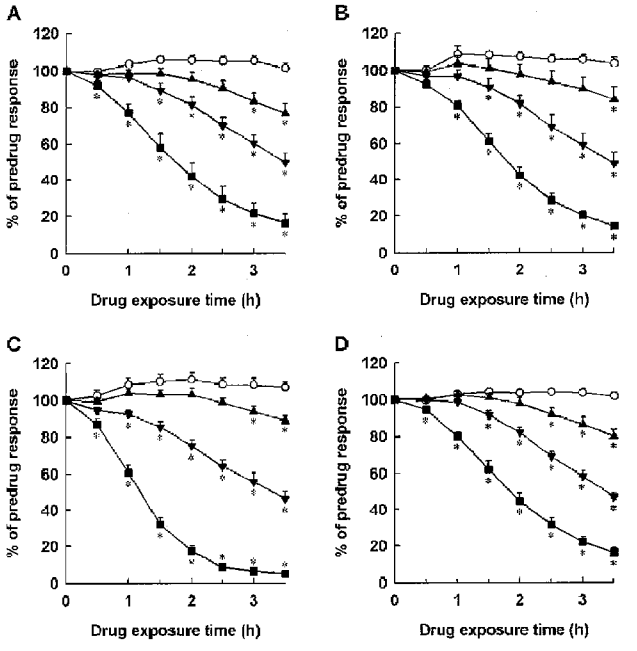

Fig. 2. Effects of R/S-(A), S-(B), R-amlodipine camsylate (C) and S-amlodipine besylate (D) on contractions of the rat aorta induced by addition of 2 mM Ca^{2+} to tissues depolarized with 45 mM K^+. For panel A, Vehicle (open circle), 1 nM (closed triangle), 3 nM (closed inverted triangle), 10 nM (closed square). For panel B and D, Vehicle (open circle), 0.5 nM (closed triangle), 1.5 nM (closed inverted triangle), 5 nM (closed square). For panel C, Vehicle (open circle), 300 nM (closed triangle), 1,000 nM (closed inverted triangle), 3,000 nM (closed square). Values are mean percentage ± S.E.M. (n = 7 – 8). $^*p < 0.05$ as compared with vehicle-treated group.

Antihypertensive effects in SHR

The effects of orally administered R/S-, S-, R-amlodipine camsylate on systolic blood pressure in conscious SHR were measured and compared with that of S-amlodipine besylate (Fig. 4). The R/S-, S-amlodipine camsylate and S-amlodipine besylate produced a dose-dependent decrease in systolic blood pressure with a slow onset of the effect, the maximal effect being reached 2-6 h after the administration. The antihypertensive effects of all drugs except for R-amlodipine significantly

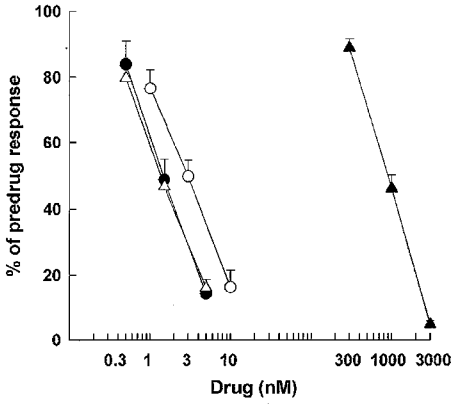

Fig. 3. Concentration-response curves for the inhibition of responses to Ca^{2+} in the rat aorta by R/S-amlodipine camsylate (open circle), S-amlodipine camsylate (closed circle), R-amlodipine camsylate (closed triangle) and S-amlodipine besylate (open triangle), derived from the 3.5 h data points (See Fig. 1). Values are mean percentage ± S.E.M. (n = 7 – 8).

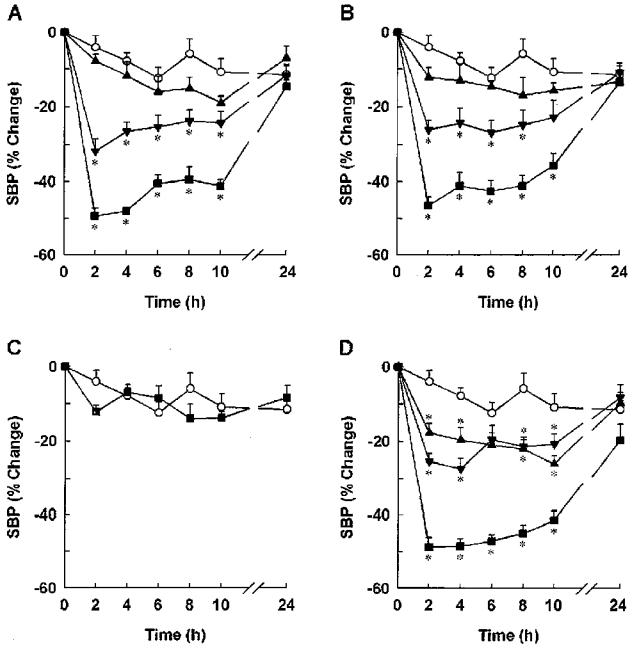

Fig. 4. Effects of R/S-(A), S-(B), R-amlodipine camsylate (C) and S-amlodipine besylate (D) on systolic blood pressure (SBP) in conscious spontaneously hypertensive rats. For panel A, Vehicle (open circle), 1 mg/kg (closed triangle), 3 mg/kg (closed inverted triangle), 10 mg/kg (closed square). For panel B and D, Vehicle (open circle), 0.5 mg/kg (closed triangle), 1.5 mg/kg (closed inverted triangle), 5 mg/kg (closed square). For panel C, Vehicle (open circle), 10 mg/kg (closed square). Values are mean percentage ± S.E.M. (n = 8). $^{*}p < 0.05$ as compared with vehicle-treated group.

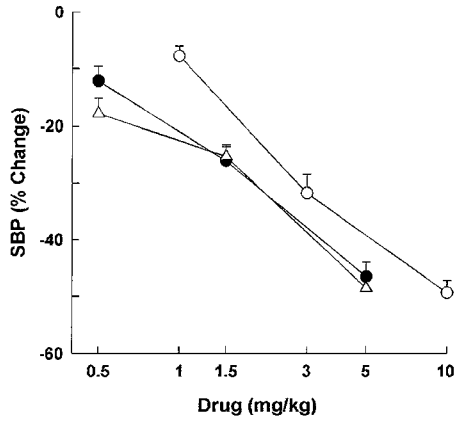

Fig. 5. Dose-response curves for the systolic blood pressure in conscious spontaneously hypertensive rats (See Fig. 4) by R/S-amlodipine camsylate (open circle), S-amlodipine camsylate (closed circle), S-amlodipine besylate (open triangle), derived from the 2 h data points. Values are mean percentage ± S.E.M. (n = 8).

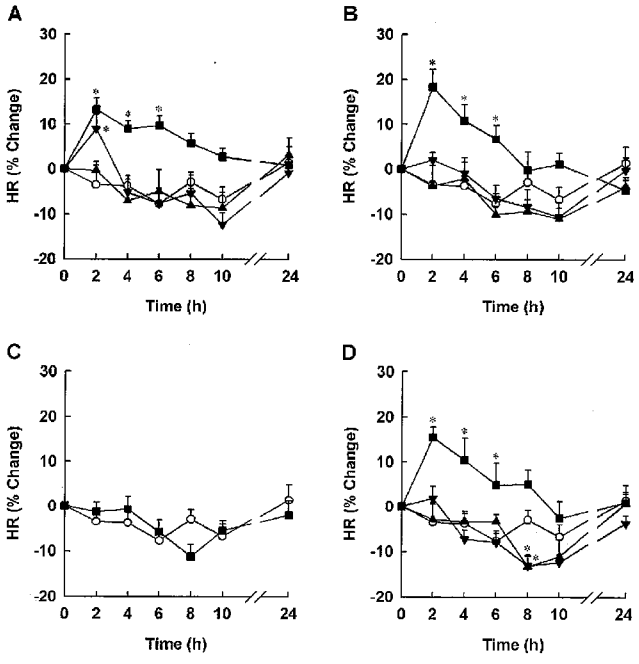

Fig. 6. Effects of R/S-(A), S-(B), R-amlodipine camsylate (C) and S-amlodipine besylate (D) on heart rate (HR) in conscious spontaneously hypertensive rats. For panel A, Vehicle (open circle), 1 mg/kg (closed triangle), 3 mg/kg (closed inverted triangle), 10 mg/kg (closed square). For panel B and D, Vehicle (open circle), 0.5 mg/kg (closed triangle), 1.5 mg/kg (closed inverted triangle), 5 mg/kg (closed square) and for panel C, Vehicle (open circle), 10 mg/kg (closed square). Values are mean percentage ± S.E.M. (n = 8). $^{*}p < 0.05$ as compared with vehicle-treated group.

persisted for more than 10 h. The maximal antihypertensive effects of S-amlodipine camsylate (-12.2 ± 2.6, -26.1 ± 2.4 and -46.6 ± 2.5% at concentration of 0.5, 1.5 and 5 mg/kg, respectively) were not significantly different with those of S-amlodipine besylate (-17.7 ± 2.5, -25.3 ± 2.1 and -48.6 ± 2.4% at concentration of 0.5, 1.5 and 5 mg/kg, respectively). In case of R/S-amlodipine camsylate, the maximal antihypertensive effect was -7.8 ± 1.8, -31.8 ± 3.3 and -49.4 ± 2.2% at concentration of 1, 3 and 10 mg/kg, respectively. As shown in Figure 5, the ED_{20} value (i.e. dose that decreased the maximal systolic blood pressure by 20%) of S-amlodipine camsylate (0.89 mg/kg) was two fold higher than that of R/S-amlodipine camsylate (1.82 mg/kg) and similar to that of S-amlodipine besylate (0.71 mg/kg). In contrast, the R-amlodipine camsylate has no effect even-though administrated high concentration 10 mg/kg.

The effects of R/S-, S-, R-amlodipine camsylate and amlodipine besylate on heart rate in conscious SHR are shown in Figure 6. All drugs except for R-amlodipine camsylate produced a dose-dependent increase in heart rate, the maximum being reached 2 h after administration. The significant increase of heart rate by both drugs was persisted for 2-6 h after admin-

istration of 10 mg/kg. The maximal effect of S-amlodipine camsylate on heart rate (18.2 ± 4.0%) was not significantly different with those of R/S-amlodipine camsylate (13.3 ± 2.5%) and S-amlodipine besylate (15.5 ± 2.3%).

DISCUSSION

The present study was performed to evaluate and compare the vasorelaxant effect and antihypertensive effect of R/S-, S- and R-amlodipine camsylate with those of S-amlodipine besylate as reference formulation.

In vasorelaxant study on isolated rat aorta, all amlodipine camsylates exhibited concentration-dependent inhibitory activity on Ca^{2+}-induced aortic contractions with very slow onset of action, the peak effect being reached 3.5 h like S-amlodipine besylate (Fig. 2). These observations are consistent with the results of a previous *in vitro* study indicating that amlodipine display a remarkably slow onset of action in contrast to nifedipine (Burges *et al.*, 1987). Among them, the S-amlodipine camsylate exhibited the most vasorelaxant potency, while R-amlodipine camsylate exhibited 590-fold lower vasorelaxant potency than S-amlodipine. These results demonstrate that S-amlodipine camsylate, and not R-amlodipine camsylate, is the cause of the vasorelaxant effects associated with racemic amlodipine camsylate. These observations were in line with previous studies (Goldmann *et al.*, 1992; Zhang *et al.*, 2002), which calcium channel blocking effect of racemic amlodipine is confined to S-amlodipine; R-amlodipine has 1000-fold less activity than its S-enantiomer. In comparing with S-amlodipine besylate, S-amlodipine camsylate elicited the similar pattern and potency. The vasorelaxant effect of S-amlodipine camsylate on Ca^{2+}-induced aortic constriction was not significantly different with that of S-amlodipine besylate (ED_{50}: 1.50 ± 0.24 and 1.44 ± 0.14 nM, respectively).

In SHR study, all amlodipine camsylates except for R-enantiomer exhibited dose-dependent anti-hypertensive effect in SHR with a very slow onset of action, the maximum being reached 2-6 h (Fig. 4). These results were consistent with previous study (Yamanaka *et al.*, 1991), which has been shown that amlodipine produces the antihypertensive effect in SHR with a profile of slow onset and long duration unlike nifedipine. As we expected, S-amlodipine camsylate also exhibited the most antihypertensive potency in SHR. Although the amount of S-amlodipine camsylate administered was only half that of R/S-amlodipine camsylate, S-amlodipine camsylate had a comparable hemodynamic profile. In contrast, the R-amlodipine camsylate has no effect although administrated high concentration. These results suggest that S-amlodipine camsylate completely contributed to the anti-hypertensive effects of R/S-amlodipine camsylate and that camsylate, conjugated for new formulation, dose not affect the anti-hypertensive activities of S-amlodipine in SHR. A comparison of the EC_{20} values obtained in SHR reveal that S-amlodipine camsylate (EC_{20}: 0.89 mg/kg) is similar potency with S-amlodipine besylate (EC_{20}: 0.71 mg/kg). These results demonstrate that in view of antihypertensive effects, both formulations have no different. In measuring of heart rate in SHR, R/S-, S-amlodipine camsylate and S-amlodipine besylate produced an increase heart rate with a similar magnitude. These results might be considered to reflex mechanism of a hemodynamic counterregulation of the decrease in blood pressure, as previously reported for other calcium channel blockers and potassium channel activators (Dodd *et al.*, 1989).

In conclusion, the present study demonstrates that the S-amlodipine camsylate has the potency and long-lasting antihypertensive activity as single enantiomer drug and its activity was comparable to that of S-amlodipine besylate, in terms of both the vasorelaxant effect on isolated rat aorta and antihypertensive effects in SHR.

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