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Antihypertensive Effect of Amlodipine Adipate, a Novel Salt of Amlodipine, in Hypertensive Rat Models

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Abstract – The vascular relaxant effect of amlodipine adipate, a new salt of amlodipine, was evaluated in isolated rat aorta, and compared with that of amlodipine besylate. Furthermore, antihypertensive effects were measured in hypertensive rat models, such as spontaneously hypertensive rats (SHR) and renal hypertensive rats (RHR). Amlodipine adipate concentration-dependently inhibited Ca^{2+}-induced contraction of rat aorta with a very slow onset of action (reached its maximum at 3.5 h; IC_{50}: 3.76 nM), having a pattern and a potency similar to those of amlodipine besylate (IC_{50}: 4.01 nM). In SHR and RHR, orally administered amlodipine adipate produced a dose-dependent and long-lasting (>10-24 h) antihypertensive effect (ED_{20}: 2.48 and 1.57 mg/kg, respectively), with a pattern and a potency similar to those of amlodipine besylate (ED_{20}: 2.50 and 1.99 mg/kg in SHR and RHR, respectively). These results suggest that amlodipine adipate is a potent and long-lasting antihypertensive agent and that its antihypertensive effect is not significantly different to that of amlodipine besylate.

Keywords □ antihypertensive effect, amlodipine, adipate, calcium antagonist, SHR, RHR

INTRODUCTION

Calcium channel blockers have proved to be useful in the treatment of cardiovascular disorders, principally hypertension and angina. A great deal of attention has focused on dihydropyridine calcium channel blockers (nifedipine), because this chemical class appears to have greater selectivity on vascular cells than on cardiac pacemaker cells or myocardial cells, as compared with the phenylalkylamine (verapamil) and benzothiazepine (diltiazem) derivatives (Cauvin *et al.*, 1983). Thus, the dihydropyridine calcium channel blockers are associated with a low incidence of cardiac depression and conduction disturbances. Although these drugs have been very extensively used, the ease of therapy has been hampered by their poor pharmacokinetic profiles, such as very high metabolic clearance rates, short elimination half-lives, and very low oral bioavailability (Abernethy, 1989)

Amlodipine is a charged dihyropyridine-type third generation calcium channel blocker which was synthesized in an attempt to develop a compound with a typical dihydropyridine pharmacologic profile, which would also have the properties of increased oral bioavailability and low clearance rates in humans. The principal difference in its chemical structure from other members of the class, including the prototype nifedipine lies in the presence of a side-chain in the 2-position of the dihydropyridine ring, which carries a basic amino group. This renders the molecule >90% ionized at physiologic pH, and this feature is believed to be primarily responsible for the marked differences in physicochemical, pharmacologic and pharmacokinetic properties displayed by amlodipine, as compared with other 1,4-dihydropyridines (Dodd *et al.*, 1989; Mathur *et al.*, 2002). The pharmacokinetic evaluation of amlodipine in dogs, mice, and rats indicated absolute bioavailability of 88, 100 and 100%, respectively, and calculated elimination half-lives of 30, 11 and 3 h, respectively (Stopher *et al.*, 1988, Burges and Moisey, 1994; Phillips *et al.*, 2003). Actually, oral pharmacokinetics after 2.5, 5 and 10 mg doses in healthy male subjects similarly indicated elimination half-lives of 31 to 37 h (Abernethy, 1989; Meredith and Elliott, 1992).

Recently, amlodipine adipate was developed by CJ Corp., as a generic salt form of amlodipine besylate. The present study was performed to evaluate the vasorelaxant effect of amlodipine adipate on isolated rat aorta, and antihypertensive effects in spontaneously hypertensive rats (SHR) and renal hypertensive rats (RHR). These results were compared with those of

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amlodipine besylate to check the bioequivalence of two drugs.

MATERIALS AND METHODS

Animals

Male SHR (13-14 weeks) and male Sprague-Dawley rats (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

Amlodipine besylate and amlodipine adipate were supplied by CJ Corp. (Seoul, Korea), and dissolved in distilled water. 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 rats 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% CO_2) 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 amlodipine besylate and amlodipine adipate 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 amlodipine besylate (1, 3 or 10 nM), amlodipine adipate (1, 3 or 10 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 the administration of the drugs.

Antihypertensive effects in SHR

The measurements of systolic blood pressure and heart rate were made by the tail-cuff method (Multichannel 8000, TSE, Kronberg, Germany) at 2, 4, 6, 8, 10 and 24 h after the administration of amlodipine besylate (1, 3 or 10 mg/kg), amlodipine adipate (1, 3 or 10 mg/kg) or vehicle (distilled water). To measure the systolic blood pressure, SHR was prewarmed at 37°C for 5-10 min in a restraining cage in a warming box (Yamanaka *et al.*, 1991; Lee *et al.*, 1998, 1999). Results were expressed as percentage change from control systolic blood pressure and heart rate.

Antihypertensive effects in RHR

RHR was prepared by the ligation of left renal artery (2-kidney, 1-ligation type RHR) as described previously (Cangiano *et al.*, 1979; Lee and Shin 1994; Lee *et al.*, 1998, 1999). Briefly, SD rats were anesthetized with ketamine·HCl (125 mg/kg, i.p.) and a small incision was made on the left side of abdomen. The left renal artery was separated from the vein near the junction with the aorta, taking care not to traumatize the vein and kidney, and then made a complete ligature with 4-0 sterile silk. In our previous studies, six to eight days after the ligation of left renal artery, a good correlation between development of hypertension and plasma renin activity has been proved (Lee and Shin 1994; Lee *et al.*, 1998, 1999), and thus rats with systolic blood pressure of more than 180 mmHg were used as renal hypertensive rats in this study. The measurements of systolic blood pressure and heart rate were made by the tail-cuff method as described in the above.

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 amlo-

dipine adipate, its effect on Ca^{2+}-induced aortic constriction was measured and compared with that of amlodipine besylate (Fig. 1). Both amlodipine besylate and amlodipine adipate concentration-dependently inhibited Ca^{2+}-induced aortic contriction with very slow action, the peak effect being reached 3.5 h at all concentrations used in this study. The maximal Ca^{2+}-induced aortic constriction of amlodipine besylate and amlodipine adipate were 86.7 ± 2.9% and 83.5 ± 3.3% at concentration of 1 nM, 64.9 ± 2.3% and 62.6 ± 3.7% at concentration of 3 nM, and 21.2 ± 1.7% and 21.2 ± 3.3% at concentration of 10 nM, respectively. The inhibitory effect of amlodipine adipate on Ca^{2+}-induced aortic constriction was not significantly different with that of amlodipine besylate at all concentrations. Furthermore, the IC_{50} value (obtained from these curves for inhibition of Ca^{2+}-induced aortic constriction) of amlodipine adipate was also similar with that of amlodipine besylate (3.76 ± 0.67 and 4.01 ± 0.55 nM, respectively).

Antihypertensive effects in SHR

The effects of orally administered amlodipine besylate and amlodipine adipate on systolic blood pressure in conscious SHR are shown in Fig 2. The predose value of systolic blood pressure in SHR was 205.4 ± 1.9 mmHg. Both amlodipine besylate and amlodipine adipate 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 both drugs significantly persisted for more than 10 h (>24 h after administration of 10 mg/kg). The maximal antihypertensive effects of amlodipine besylate and amlodipine adipate were -8.8 ± 1.7% and -8.0 ± 1.5% at dose of 1 mg/kg, -20.6 ± 1.5% and -22.3 ± 2.8% at dose of 3 mg/kg, and -41.1 ± 2.0% and -41.7 ± 1.6% at dose of 10 mg/kg, respectively. The antihypertensive effect of amlodipine adipate was not significantly different with that of amlodipine besylate at all doses. The ED_{20} value (dose that decreased the maximal systolic blood pressure by 20%) of amlodipine adipate was also similar to that of amlodipine besylate (2.48 ± 0.44 and 2.50 ± 0.42 mg/kg, respectively). The effects of amlodipine besylate and amlodipine adipate on the heart rate in conscious SHR are shown in Fig 3. The predose value of the heart rate in SHR was 374.3 ± 3.9 beats/min. Both amlodipine besylate and amlodipine adipate produced a dose-dependent increase in the heart rate, the maximum being reached in 2 h after administration. The significant increase of the heart rate by both drugs was persisted for 10 h after administration of 10 mg/kg. The maximal effect of amlodipine adipate on the heart rate was not significantly different with that of amlodipine besylate at the doses tested.

Antihypertensive effects in RHR

The effects of orally administered amlodipine besylate and amlodipine adipate on systolic blood pressure in conscious RHR are shown in Fig 4. The predose value of systolic blood pressure in RHR was 203.7 ± 1.7 mmHg. Both amlodipine besylate and amlodipine adipate produced a dose-dependent decrease in systolic blood pressure with a slow onset of action, the maximum being reached in 2-6 h after the administration. The maximal antihypertensive effects of amlodipine besylate and amlodipine adipate were -14.7 ± 1.4% and -16.1 ± 1.0% at dose of 1 mg/kg, -28.1 ± 4.6% and -28.9 ± 0.9% at dose of 3 mg/kg, and -40.9 ± 3.1% and -39.8 ± 2.5% at dose of 10 mg/kg, respectively, without significant differences between groups administered with amlodipine besylate and amlodipine adipate.

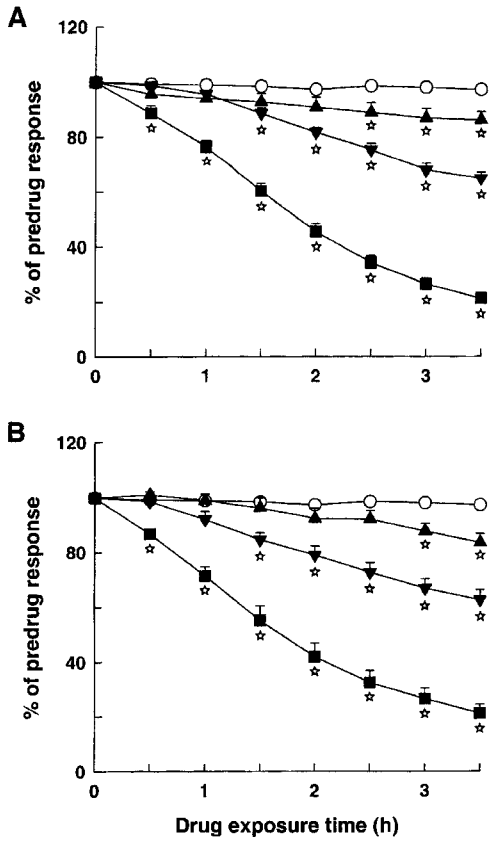

Fig. 1. Effects of amlodipine besylate (A) and amlodipine adipate (B) on contractions of the rat aorta induced by addition of 2 mM Ca^{2+}. Vehicle (open circle, n=14), 1 nM (closed triangle, n=6), 3 nM (closed inverted triangle, n=6), 10 nM (closed square, n=6). Values are mean percentage ± S.E.M. $^{\star}p < 0.05$ as compared with the value before administration.

The ED_{20} value of amlodipine adipate was also similar to that of amlodipine besylate (1.57 ± 0.39 and 1.99 ± 0.78 mg/kg, respectively). The effects of amlodipine besylate and amlodipine adipate on the heart rate in conscious RHR are shown in Fig 5. The predose value of the heart rate in RHR was 454.6 ± 6.8 beats/min. Both amlodipine besylate and amlodipine adipate produced a dose-dependent increase in the heart rate, the maximum being reached in 2 h after administration. The significant increase of the heart rate by both drugs was persisted for 10 h after administration of 10 mg/kg, respectively.

DISCUSSION

The present study was performed to evaluate the vasorelaxant effect of amlodipine adipate on isolated rat aorta, and antihypertensive effects in SHR and RHR, which was compared with those of amlodipine besylate. Amlodipine besylate concentration-dependently inhibited Ca^{2+}-induced contractions of depolarized rat aorta with very slow onset of action, the peak effect being reached in 3.5 h. These observations are in line 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). Amlodipine adipate treatment also produced a pattern and a potency similar to those seen in amlodipine besylate treatment. The inhibitory effect of amlodipine adipate on Ca^{2+}-induced aortic constriction was not significantly different with that of amlodipine besylate at each concentration (IC_{50} : 3.76 ± 0.67 and 4.01 ± 0.55 nM, respectively).

Previously, it has been shown that amlodipine produces the antihypertensive effect in SHR with a potency similar to nifedipine but with a profile of slow onset and long duration (Yamanaka *et al.*, 1991). This is in agreement with results of the present study, which found that amlodipine besylate produced a dose-dependent decrease in blood pressure in SHR and RHR with a very slow onset of action, the maximum being

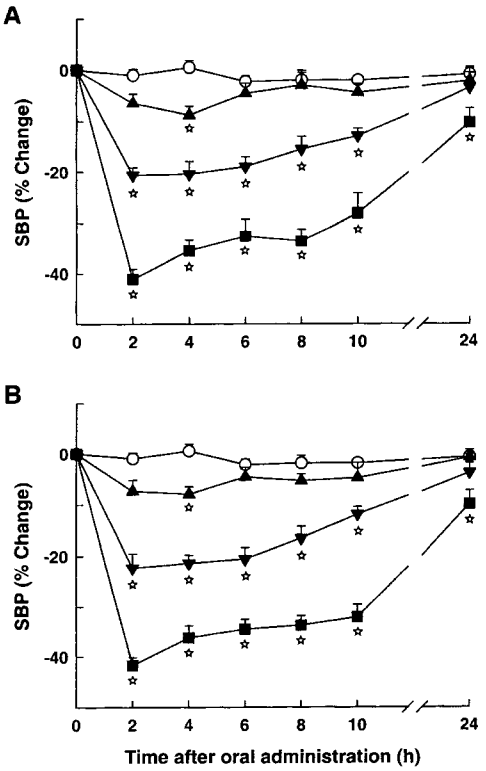

Fig. 2. Effects of amlodipine besylate (A) and amlodipine adipate (B) on systolic blood pressure (SBP) in conscious spontaneously hypertensive rats. Vehicle (open circle, n=7), 1 mg/kg (closed triangle, n=7), 3 mg/kg (closed inverted triangle, n=7 for panel A, n=8 for panel B), 10 mg/kg (closed square, n=8). Values are mean percentage ± S.E.M. $^{\star}p < 0.05$ as compared with the value before administration.

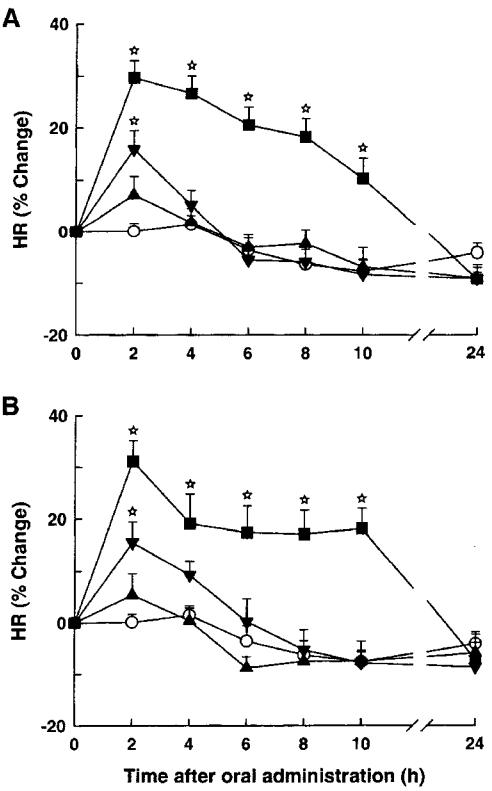

Fig. 3. Effects of amlodipine besylate (A) and amlodipine adipate (B) on heart rate (HR) in conscious spontaneously hypertensive rats. Vehicle (open circle, n=7), 1 mg/kg (closed triangle, n=7), 3 mg/kg (closed inverted triangle, n=7 for panel A, n=8 for panel B), 10 mg/kg (closed square, n=8). Values are mean percentage ± S.E.M. $^{\star}p < 0.05$ as compared with the value before administration.

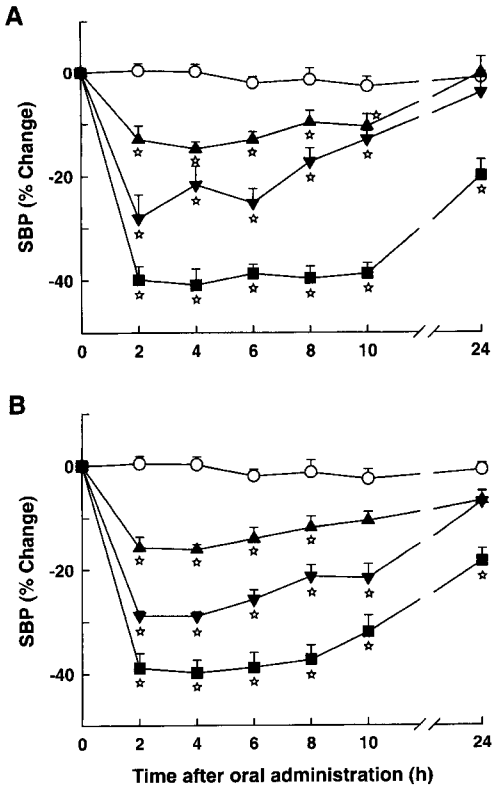

Fig. 4. Effects of amlodipine besylate (A) and amlodipine adipate (B) on systolic blood pressure (SBP) in conscious renal hypertensive rats. Vehicle (open circle, n=7), 1 mg/kg (closed triangle, n=6 for panel A, n=7 for panel B), 3 mg/kg (closed inverted triangle, n=6 for panel A, n=7 for panel B), 10 mg/kg (closed square, n=7). Values are mean percentage ± S.E.M. $^{\star}p < 0.05$ as compared with the value before administration.

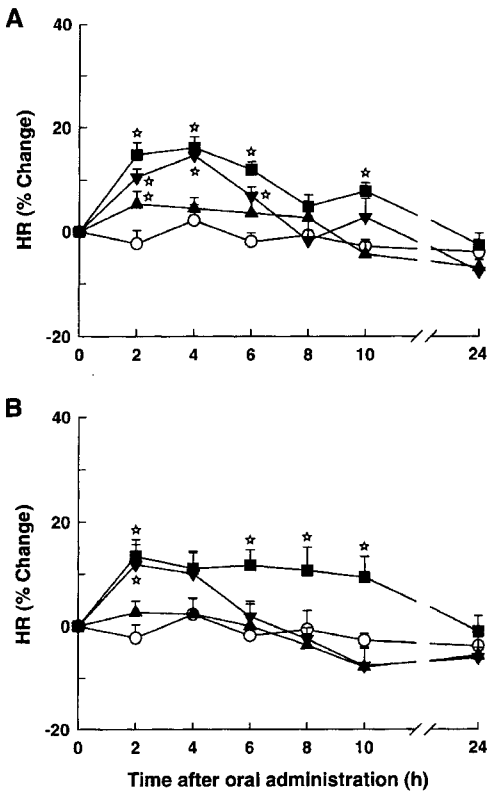

Fig. 5. Effects of amlodipine besylate (A) and amlodipine adipate (B) on heart rate (HR) in conscious renal hypertensive rats. Vehicle (open circle, n=7), 1 mg/kg (closed triangle, n=6 for panel A, n=7 for panel B), 3 mg/kg (closed inverted triangle, n=6 for panel A, n=7 for panel B), 10 mg/kg (closed square, n =7). Values are mean percentage ± S.E.M. $^{\star}p < 0.05$ as compared with the value before administration.

reached 2-6 h. An oral administration of amlodipine adipate in SHR (and RHR) produced a pattern and potency similar to those seen in administration of amlodipine besylate. The antihypertensive effect of amlodipine adipate was not significantly different with that of amlodipine besylate at each doses (ED_{20} : 2.48 ± 0.44 and 2.50 ± 0.42 mg/kg in SHR, 1.57 ± 0.39 and 1.99 ± 0.78 mg/kg in RHR, respectively). In SHR and RHR, both amlodipine besylate and amlodipine adipate produced an increase in heart rate with a similar magnitude. As previously reported for other calcium channel blockers and potassium channel activators, it might be due to reflex mechanism of a hemodynamic counterregulation of the decrease in blood pressure (Dodd *et al.*, 1989). Taken together, these results suggest that amlodipine adipate is a potent and long-lasting antihypertensive agent with slow onset of action, and its antihypertensive effect is not significantly different to that of amlodipine besylate.

REFERENCES

Abernethy, D. R. (1989). The pharmacokinetic profile of amlodipine. *Am. Heart J.* **118**, 1100-1103.

Burges, R. A., Gardiner, D. G., Gwilt, M., Higgins, A. J., Blackburn, K. J., Campbell, S. F., Cross, P. E. and Stubbs, J. K. (1987). Calcium channel blocking properties of amlodipine in vascular smooth muscle and cardiac muscle in vitro: evidence for voltage modulation of vascular dihydropyridine receptors. *J. Cardiovasc. Pharmacol.* **9**, 110-119.

Burges, R. and Moisey, D. (1994). Unique pharmacologic properties of amlodipine. *Am. J. Cardiol.* **73**, 2A-9A.

Cangiano, J. L., Rodriguez-Sargent, C. and Martinez-Maldonado, M. (1979). Effects of antihypertensive treatment on systolic blood pressure and renin in experimental hypertension in rats. *J. Pharmacol. Exp. Ther.* **208**, 310-313.

Cauvin, C., Loutzenhiser, R. and Van Breemen C. (1983). Mechanisms of calcium antagonist-induced vasodilation. *Annu. Rev. Pharmacol. Toxicol.* **23**, 373-396.

Dodd, M. G., Gardiner, D. G., Carter, A. J., Sutton, M. R. and Burges, R. A. (1989). The hemodynamic properties of amlo-

dipine in anesthetised and conscious dogs: comparison with nitrendipine and influence of beta-adrenergic blockade. *Cardiovasc. Drugs Ther.* **3**, 545-555.

Lee, B. H. and Shin, H. S. (1994). In vivo pharmacological evaluation of newly synthesized nonpeptidic AT1 receptor antagonists in rats. *Arch. Pharm. Res.* **17**, 263-268.

Lee, B. H., Yoo, S. E. and Shin, H. S. (1998). Hemodynamic profile of SKP-450, a new potassium-channel activator. *J. Cardiovasc. Pharmacol.* **31**, 85-94.

Lee, B. H., Seo, H. W., Kwon, K. J., Yoo, S. E. and Shin, H. S. (1999). In vivo pharmacologic profile of SK-1080, an orally active nonpeptide AT1-receptor antagonist. *J. Cardiovasc. Pharmacol.* **33**, 375-382.

Lee, B. H., Seo, H. W., Yoo, S. E., Kim, S. O., Lim, H. and Shin, H. S. (2001). Differential action of KR-31378, a novel potassium channel activator, on cardioprotective and hemodynamic effects. *Drug Dev. Res.* **54**, 182-190.

Mathur, S., Syme, H., Brown, C. A., Elliot, J., Moore, P. A., Newell, M. A., Munday, J. S., Cartier, L. M., Sheldon, S. E. and Brown, S. A. (2002). Effects of the calcium channel antagonist amlodipine in cats with surgically induced hypertensive renal insufficiency. *Am. J. Vet. Res.* **63**, 833-839.

Meredith, P. A. and Elliott, H. L. (1992). Clinical pharmacokinetics of amlodipine. *Clin. Pharmacokinet.* **22**, 22-31.

Phillips, R. A., Kloner, R. A., Grimm, R. H. Jr. and Weinberger, M. (2003). The effects of amlodipine compared to losartan in patients with mild to moderately severe hypertension. *J. Clin. Hypertens. (Greenwich)* **5**, 17-23.

Shin, H. S., Seo, H. W., Yoo, S. E. and Lee, B. H. (1998). Cardiovascular pharmacology of SKP-450, a new potassium channel activator, and its major metabolites SKP-818 and SKP-310. *Pharmacology* **56**, 111-124.

Stopher, D. A., Beresford, A. P., Macrae, P. V. and Humphrey, M. J. (1988). The metabolism and pharmacokinetics of amlodipine in humans and animals. *J. Cardiovasc. Pharmacol.* **12**, S55-S59.

Yamanaka, K., Suzuki, M., Munehasu, S. and Ishiko, J. (1991). Antihypertensive effects of amlodipine, a new calcium antagonist. *Nippon Yakurigaku Zasshi* **97**, 115-126.