Antihypertensive Effects of CS-905, a Novel Dihydropyridine Ca⁺⁺ Channel Blocker

Kiyoshi OIZUMI, Hiroshi NISHINO, Hiroyuki KOIKE*,
Toshio SADA, Masaaki MIYAMOTO and Tomio KIMURA¹

Sankyo Biological Research Laboratories, Sankyo Co., Ltd.,
1-2-58, Hiromachi, Shinagawa-ku, Tokyo 140, Japan

¹Pharmaceutical Research Department Ube Laboratory, Ube Industries, Ltd.,
1978-5 Kogushi, Ube, Yamaguchi 755, Japan

Accepted May 22, 1989

Abstract—CS-905 is a novel dihydropyridine calcium blocker. A single oral administration of CS-905 or nifedipine at doses of 0.3–3.0 mg/kg produced a dose-dependent reduction of blood pressure in conscious SHR. CS-905, when administered orally in conscious SHR, was more than 3 times as potent as nifedipine. Unlike the hypotensive effect of nifedipine, that of CS-905 has a gradual onset and is long-lasting, with little increase in heart rate. An intravenous administration of CS-905 also produced a hypotension with a slow onset and long duration in SHR, but CS-905 was 3 times less potent than nifedipine by intravenous administration. This difference may be attributed to the first pass effect, which was associated with nifedipine but not with CS-905. The blood pressure lowering effects of CS-905 was most potent in DOCA-salt hypertensive rats, followed by SHR, RHR and normotensive rats, in this order. CS-905 is expected to be an antihypertensive agent that is effective on a once a day regimen in clinical settings.

Dihydropyridine calcium blockers, represented by nifedipine and nicardipine, are widely used for the treatment of hypertensive disorders (1–5). However the clinical usefulness of these calcium blockers has been limited by dosage frequency because their durations of action are relatively short (6). In addition, these compounds induce an acute fall in blood pressure which is accompanied by a counterregulatory increase in heart rate (1, 2, 7).

To eliminate some of these problems, we have synthesized dihydropyridine derivatives and assayed them using conscious SHR, with special attention to the onset and duration of the antihypertensive effect. We describe here the antihypertensive actions of one of these compounds, CS-905, whose chemical structure is shown in Fig. 1. Whereas all the existing dihydropyridine calcium blockers have two methyl groups located at positions

Fig. 1. Chemical structure of CS-905. (±)-3-(1-Diphenylmethylazetidin-3-yl)-5-isopropyl 2-amino-1,4-dihydro-6-methyl-4-(m-nitrophenyl)-3,5-pyridine-dicarboxylate.

2 and 6 of the dihydropyridine ring, one methyl group is substituted for by an amino group in the CS-905 molecule. The agent is not water soluble and resistant to light decomposition.

Materials and Methods

Antihypertensive effects in conscious SHR

Male SHR, 23 weeks old, were anesthetized with sodium pentobarbital at the dose

* To whom reprint request should be addressed.

of 30 mg/kg, i.p. The animal was surgically inserted with an aortic cannula via the left femoral artery for measuring blood pressure and heart rate. For the experiments with intravenous administration, the animal was also cannulated into the femoral vein for the injection of drugs. The other ends of the cannulae were led under the skin and exteriorized at the back of the neck. The rat was placed in an individual cage after surgery and allowed free access to tap water but not to food. When the rats recovered from surgical stress, normally 2–3 days after surgery, the aortic cannula was connected to a pressure transducer, and blood pressure and heart rate were continuously recorded with a blood pressure measuring system that was developed in our laboratories. After blood pressure and heart rate were stabilized, a single oral dose of CS-905 or nicardipine or a single intravenous dose of CS-905 or nicardipine was given. Blood pressure and heart rate were monitored for up to 24 hr after administration.

Preparation of hypertensive rats

In addition to 14-week-old normotensive Wistar Imamichi rats, the following hypertensive models were prepared and used in the same manner as SHR for the evaluation of antihypertensive effects of CS-905.

1) Renal hypertensive rats (RHR): Wistar Imamichi rats weighing 130–180 g, 10 weeks old, were used. Renal hypertension was induced by placing silver clip with an internal split distance of 0.225 mm around the left renal artery. The right renal artery was left intact. The thus made hypertensive rat was designated as the 1-clip-2-kidney renal hypertensive rat. Eight weeks after this procedure, the animals were cannulated, and experiments were performed in conscious state as described above.

2) DOCA salt hypertensive rats (DOCA salt): A left nephrectomy was performed on 10-week-old male Wistar Imamichi rats that weighed about 100 g. One week after the left nephrectomy, animals were injected weekly with deoxycorticosterone acetate (Sigma, in 0.3% CMC solution) and given 1% saline as drinking water. Six weeks after DOCA injection when the mean blood pressure reached about 190 mmHg, the animals were cannulated, and experiments were performed in the conscious

state as described above.

Antihypertensive effects in anesthetized SHR

Male SHR, 23 weeks old, were anesthetized with Inactin at 100 mg/kg, i.p. The animal was cannulated into the left femoral artery and vein for measuring blood pressure and injecting drugs, respectively. For injecting drugs into the portal vein, a median abdominal incision was performed and a needle connected to polyethylene cannulae was inserted into a mesenteric vein. After the blood pressure became stabilized, experiments were performed.

Isolated vascular preparations

Spirally-cut aortae from male Wistar Imamichi rats (300–350 g) were suspended in Krebs-Henseleit solution (144.8 mM Na⁺, 5.9 mM K⁺, 2.5 mM Ca²⁺, 1.2 mM Mg²⁺, 25.0 mM HCO₃⁻, 1.2 mM SO₄²⁻, 1.2 mM H₂PO₄⁻, 129.5 mM Cl⁻ and 11.1 mM glucose) maintained at 36.5±0.5°C and bubbled with 95% O₂ : 5% CO₂. Their isometric tension was measured with a force-displacement transducer (TB-612T, Nihon Kohden) connected to a carrier amplifier (AP-601G, Nihon Kohden). The muscle tension was recorded on a thermal-pen-writing recorder (RJG-4128, Nihon Kohden). Initial tension was set at 1 g, and the tissues were allowed to equilibrate for 1 hr.

In one series of experiments, the rate of onset for CS-905 and nicardipine to inhibit K⁺-induced contractions was determined as follows: Contractions to 40 mM K⁺ were evoked at 60 min intervals, each cycle comprising a 25 min exposure to K⁺ followed by a washout with fresh solution and a 35 min recovery. After two successive contractions of an equal size were obtained as controls, CS-905 (1, 3 or 10 nM), nicardipine (0.3 or 1 nM) or vehicle (N,N-dimethylformamide 0.067% vol./vol.) was added 15 min before the next application of K⁺. The high K⁺-challenge was instituted again under the presence of drugs at an 1 hr interval up to 6 hr.

In another series of experiments, we investigated the reversal of K⁺ contractions after the drug was washed out (washout experiment). After two control responses to high K⁺ were obtained, CS-905 (3, 10 or 30 nM), nicardipine (1 or 3 nM) or vehicle was added to the bathing solution 15 min before

the next addition of K^+ . The solution was switched to the normal solution 25 min after addition of high K^+ ; the tissue was thus exposed to the drug for 40 min. High K^+ contractions were evoked at 65–75 min intervals up to 250 min after the tissue was washed to follow the time course for the recovery after drug removal.

Drugs

CS-905 and nicardipine were suspended in 0.3% CMC solution for oral administration. For intraportal venous or intravenous administration, CS-905 and nicardipine were dissolved in DMF and diluted with physi-

ological saline.

Results

Figure 2 shows the time course of percent changes in mean blood pressure (MBP) and heart rate (HR) after a single oral administration of CS-905 and nicardipine in conscious SHR. Both agents lowered blood pressure in a dose-dependent manner, but there were clear differences in the onset and the duration of action between CS-905 and nicardipine. Nicardipine produced a rapid decrease of MBP that was accompanied by a great increase in HR, and the durations of

Fig. 2. Time course for the percent changes in mean blood pressure (MBP) and heart rate (HR) after a single oral administration of CS-905 (A) or nicardipine (B) in conscious SHR. MBP and HR before administration of CS-905 were 187 ± 3 mmHg and 307 ± 5 beats/min, respectively. MBP and HR before administration of nicardipine were 189 ± 3 mmHg and 309 ± 6 beats/min, respectively. Values are means $\pm$ S.E. from six rats.

hypotension were relatively short. On the other hand, CS-905 produced a gradual and long-lasting reduction of MBP with little increase in HR. The sharp increase of HR seen immediately after administration of CS-905 was due to stress given when the drug was orally administered. In fact, the degree of initial tachycardia did not vary with the dose of CS-905 (Fig. 2A), whereas the sharp increase of HR following nicardipine became greater when the dose was increased (Fig. 2B). The magnitude of the maximum fall in BP produced by CS-905 at 0.3 and 1.0 mg/kg

nearly equalled that produced by nicardipine at 1 and 3 mg/kg, respectively. These data suggest that CS-905 was approximately 3 times as potent as nicardipine by oral administration.

CS-905 produced a long-lasting decrease of blood pressure in conscious RHR at doses of 1 and 3 mg/kg, p.o. (Fig. 3A). The antihypertensive action in RHR was less than that in SHR (Figs. 2A and 3A). In DOCA salt hypertensive rats, whose initial BP was similar to those of SHR and RHR, CS-905 produced a more marked hypotension than in SHR

Fig. 3. Time course for the percent changes in mean blood pressure (MBP) after a single oral administration of CS-905 in conscious renal hypertensive rats (RHR), DOCA salt hypertensive rats (DOCA) or normotensive rats (NR). MBP before administration in RHR (A), DOCA (B) and NR (C) were 190 ± 4 , 186 ± 5 and 107 ± 3 mmHg, respectively. Values are means $\pm$ S.E. from five rats.

Fig. 4. Dose-response relations for antihypertensive action of CS-905 in conscious DOCA-salt hypertensive rats (DOCA), SHR, renal hypertensive rats (RHR) and normotensive rats (NR). The hypotensive area was calculated as the area under the blood pressure curve during the 24 hr observation period.

(Fig. 3B). The antihypertensive action of CS-905 in normotensive rats was approximately 10 times less potent than that in SHR (Figs. 2A and 3C). Figure 4 shows the dose-response relation of CS-905 in different animal models of hypertension. The antihypertensive action was expressed as the area under the blood pressure curve during the 24 hr observation period. The magnitude of the hypotensive area produced by CS-905 was in the following order: DOCA salt > SHR > RHR > NR.

CS-905 or nicardipine was intravenously administered in conscious SHR (Fig. 5). A single intravenous administration of nicardipine produced a rapid fall of MBP, whereas that of CS-905 produced a gradual and sustained fall of MBP. The magnitudes of the maximum fall in MBP produced by CS-905 at 100 and 300 μ g/kg were similar to those produced by nicardipine at 30 and 100 μ g/kg, respectively. These data suggest that CS-905 was 3 times less potent than nicardipine by intravenous administration.

Single intraportal venous and intravenous administrations of CS-905 at 30 μ g/kg produced similar hypotensions in anesthetized SHR (Fig. 6). In contrast, a single intraportal venous administration of nicardipine produced a much smaller reduction of blood pressure than that produced by intravenous

Fig. 5. Changes in mean blood pressure after a single intravenous administration of nicardipine or CS-905 in conscious SHR. Values are means $\pm$ S.E. from six rats.

Fig. 6. Changes in mean blood pressure after a single intraportal vein and intravenous administration of nicardipine or CS-905 in anesthetized SHR. Values are means $\pm$ S.E. from five rats.

administration of the agent. These data suggest that nicardipine, but not CS-905, underwent the hepatic first pass effect.

Figure 7 (A and B) shows the time-course for inhibition of the contractions to 40 mM K⁺ in rat aorta when CS-905 and nicardipine were acutely applied. Both agents inhibited K⁺ induced contraction of rat aorta, but there was a marked difference between these agents in the rate of onset of the action. The inhibitory action occurred rapidly with nicardipine and remained constant within 75 min after the drug was added (Fig. 7B). On the

Fig. 7. Time course for inhibition of the contractile response to 40 mM K⁺ in rat aorta after adding CS-905 or nicardipine to the bathing solution. Values are means $\pm$ S.E. from three rats.

other hand, the inhibition by CS-905 developed very slowly and did not reach a stable level within the 6-hr observation period (Fig. 7A). We therefore could not compare accurately the *in vitro* potencies of the two drugs. Percent inhibition after the 6-hr incubation period indicates that CS-905 is 3–10 times less potent than nicardipine. The inhibition of K⁺ contraction produced by 4-hr incubation with CS-905 at 3 nM was abolished when the Ca²⁺ concentration in the bathing solution was raised from 2.5 mM to 25 mM, suggesting that the inhibition was due to a blockade of Ca²⁺ entry (data not shown).

Figure 8 (A and B) shows the time course for elimination of inhibition of K⁺ contractions in rat aorta after washout of CS-905 and

Fig. 8. Time course for recovery of 40 mM K^+ contraction after the removal of drugs. The tissues were exposed to CS-905 or nicardipine for 40 min and washed by drug-free Krebs-Henseleit solution. Values are means $\pm$ S.E. from three rats

nicardipine. A 40-min exposure to nicardipine (1 or 3 nM) markedly inhibited the contractile response to 40 mM K^+ . The K^+ -contraction increased gradually after nicardipine was removed from the bathing solution, and a full sized K^+ -contraction was obtained within 250 min of washout (Fig. 8B). In contrast, the 40-min exposure to CS-905 (3, 10 or 30 nM) resulted in a moderate inhibition of K^+ contraction. After washout of CS-905, the contractile response to K^+ did not recover but further declined throughout the 6-hr observation period.

Discussion

CS-905 administered orally produced a dose-dependent fall of blood pressure in normotensive and three types of hypertensive rats: SHR, RHR and DOCA salt hypertensive

rats (Fig. 4). The antihypertensive effects of Ca channel antagonists are primarily based on inhibition of transmembrane Ca influx through the voltage-dependent channels of vascular smooth muscles (8, 9). Arteries of DOCA salt hypertensive rats have been shown to have a greater responsiveness to calcium than those of normotensive rats (10). Holloway and Bohr have found that the transmembrane permeability to Ca in the femoral artery of hypertensive rats differs from that of normotensive rats (11). These reports suggest that one of the major factors contributing to the development of hypertension is an increase in transmembrane permeability to Ca . The degree of the antihypertensive effect produced by a Ca blocker in an animal model would vary with the extent to which the increased Ca permeability of vascular smooth muscle contributes to the maintenance of hypertension in that particular model. The degree of the antihypertensive effects produced by CS-905 was in the following order: DOCA salt $>$ SHR $>$ RHR $>$ NR (Fig. 4). Though further studies are needed to clarify the exact mechanisms, one of the reasons for these different antihypertensive effects may be the difference in the Ca -dependency in the genesis and maintenance of high blood pressure among these animal models.

It is well-known that nifedipine produces tachycardia both in clinical and experimental situations. This tachycardia is most probably due to baroreceptor reflexes caused by the acute fall of blood pressure (1, 2, 7), because the tachycardia by nifedipine is reduced by prior administration of propranolol (12). In the present study, nicardipine produced an acute hypotension accompanied by a sharp increase in HR, but a single oral administration of CS-905 did not increase HR (Fig. 2). The lack of tachycardia with CS-905 may partly be attributed to the gradual onset of its blood pressure lowering effects because the degree of tachycardia in baroreceptor reflexes is determined not only by the degree of hypotension but also by the rate of blood pressure fall (13).

There are two possible mechanisms that account for the gradual onset of CS-905: first, the absorption of CS-905 from the gastro-intestinal tract is slow (K. Nakamura

et al., unpublished data); secondly, the association rates of this compound with the tissue or receptor is slow. The gradual onset of hypotension even after intravenous administration of CS-905 suggest that the second mechanism is the case. Indeed, Ca^{++} -channel blockade with CS-905 developed very slowly in the isolated vascular preparations as well (Fig. 7). According to the membrane approach theory (14), due to the hydrophobic group (diphenylmethylazetidin) at position 3, the CS-905 molecules can only slowly travel in the cell membrane to reach the Ca^{++} -channels.

CS-905, unlike nicardipine, produced long-lasting antihypertensive effects. A possible explanation for this long-lasting action of CS-905 is as follows: CS-905 is bound firmly to the calcium channels, and the dissociation rate from calcium channels may be very slow. The results of washout experiments in vitro support this possibility (Fig. 8).

CS-905 was 3 times more potent than nicardipine on oral administration and was 3 times less potent on intravenous administration. This difference may be attributed to the difference in the hepatic decomposition of these two agents. Indeed, the antihypertensive action of nicardipine administered into the portal vein was much smaller than that after intravenous administration at the same dose (Fig 6). In contrast, CS-905 produced similar hypotensions by intravenous and intraportal venous administrations (Fig. 6). These results indicate that nicardipine undergoes hepatic metabolism (the first pass effect) more easily than CS-905.

In conclusion, CS-905 is a potent and long-lasting Ca channel antagonist. By oral administration in conscious SHR, CS-905 was more than three times as potent as nicardipine and its onset of action was gradual, hence producing little increase in HR. The agent produced a hypotension that was greater and longer in hypertensive rats, including DOCA-salt, SHR, and RHR, than normotensive rats. CS-905 is expected to be an antihypertensive agent that is effective on a once a day regimen in the clinical situation.

Acknowledgments: The authors gratefully acknowledge Misses Keiko Ikeda and Junko Shimoyama and Mrs. Toshiko Kumagae for their excellent

technical assistance.

References

- 1 Guazzi, M., Olivari, M.T., Polese, A., Fiorentini, C., Margrini, F. and Moruzzi, P.: Nifedipine, a new antihypertensive with rapid action. *Clin. Pharmacol. Ther.* **22**, 528–532 (1977)
- 2 Pedersen, O.L.: Calcium blockade in arterial hypertension. *Hypertension* **5**, Supp. 2, 74–79 (1983)
- 3 Thibonnier, M., Bonnet, F. and Corvol, P.: Antihypertensive effect of fractionated sublingual administration of nifedipine in moderate essential hypertension. *Eur. J. Clin. Pharmacol.* **17**, 161–164 (1980)
- 4 Bellet, M., Loria, Y. and Lallemant, A.: First-step treatment of mild to moderate uncomplicated essential hypertension by a new calcium antagonist: nicardipine. *J. Cardiovasc. Pharmacol.* **7**, 1149–1153 (1985)
- 5 Asplund, J.: Nicardipine hydrochloride in essential hypertension—a controlled study. *Br. J. Clin. Pharmacol.* **20**, 120S–124S (1985)
- 6 Forette, F., Bellet, M., Henry, J.F., Hervy, M.P., Poyard-Salmeron, C., Bouchacourt, P. and Guerret, M.: Effect of nicardipine in elderly hypertensive patients. *Br. J. Clin. Pharmacol.* **20**, 125S–129S (1985)
- 7 Pruneau, D. and Roy, F.: Comparative antihypertensive effects of nifedipine and nicardipine in adult and aged spontaneously hypertensive rats. *Arzneimittelforschung* **37**, 416–418 (1987)
- 8 Fleckenstein, A.: Specific pharmacology of calcium in myocardium, cardiac pacemakers, and vascular smooth muscle. *Annu. Rev. Pharmacol. Toxicol.* **17**, 149–166 (1977)
- 9 Schwartz, A. and Triggle, D.J.: Cellular action of calcium channel blocking drugs. *Annu. Rev. Med.* **35**, 325–339 (1984)
- 10 Hinke, J.: Effect of Ca upon contractility of small arterioles from DCA hypertensive rats. *Circ. Res.* **18**, Supp. I, 23–34 (1966)
- 11 Holloway, E.T. and Bohr, D.F.: Reactivity of vascular smooth muscle in hypertensive rats. *Circ. Res.* **33**, 678–685 (1973)
- 12 Hiwatari, M., Satoh, K. and Taira, N.: Antihypertensive effect of nifedipine on conscious renal-hypertensive dogs. *Arzneimittelforschung* **29**, 256–260 (1979)
- 13 Ruch, T.C. and Patton, H.D.: Control of arterial blood pressure. *In* Physiology and Biophysics, Edited by Scher, A.M., p. 660–683, W.B. Saunders, Philadelphia (1965)
- 14 Rhodes, D.G., George Sarmiento, J. and Herbette, L.G.: Kinetics of binding of membrane-active drugs to receptor sites. *Mol. Pharmacol.* **27**, 612–623 (1985)