HYPOTENSIVE ACTION OF DILTIAZEM IN CONSCIOUS RENAL HYPERTENSIVE DOGS: COMPARISON WITH NIFEDIPINE AND INTERACTION WITH PINDOLOL*

HIROSHI NARITA, HIDEO YABANA, YOSHIHISA SASO,* TAKEHIKO SUZUKI, MASAKAZU MINOBE** AND TAKU NAGAO*

*Biological Research Laboratory, * Tanabe Seiyaku Co., Ltd., 2-2-50, Toda-shi, Saitama, 335, Japan and Product Formulation Research Laboratory, ** Tanabe Seiyaku Co., Ltd. 3-16-89, Kashima, Yodogawa-ku, Osaka-shi, Osaka, 532, Japan*

(Received December 12, 1985)

The hypotensive action of diltiazem by oral administration to conscious renal hypertensive dogs (one kidney, one figure 8) was studied and the effect was compared with that of nifedipine. Diltiazem decreased mean blood pressure 10–20 mmHg at doses of 1–4 mg/kg. The same doses of nifedipine exhibited hypotensive actions similar to diltiazem, but nifedipine induced a more pronounced reflex tachycardia than diltiazem. Combined administration of diltiazem with pindolol produced a greater hypotension than that caused by individual drugs and caused an increase in heart rate, smaller than by pindolol alone and larger than by diltiazem alone. When 60 mg of diltiazem was administered 3 times a day for 10 consecutive days, blood pressure decreased 15 mmHg on the third day or later. Although the time course of plasma level of diltiazem on the last day was similar to that on the first day, the heart rate initially increased slightly and decreased later. Prolongation of the PQ interval of an electrocardiogram was diminished after the fourth day. In conclusion, diltiazem decreased blood pressure of renal hypertensive dogs at doses comparable to those used for clinical treatment in acute and chronic experiments.

Keywords — conscious dog; renal hypertension; diltiazem; nifedipine; pindolol; combined administration; consecutive administration; plasma level

INTRODUCTION

Recently, the Ca antagonists have received clinical attention as a new type of antihypertensive vasodilators. Among representative Ca antagonists, diltiazem and 1,4-dihydropyridine derivatives are used as antihypertensive agents in Japan.

Vasodilators are often combined with beta-blockers for the treatment of hypertensive patients. Combined effects of Ca antagonists with propranolol were studied in normotensive dogs and significant participation of the autonomic nervous system in the response to Ca antagonists was reported.¹⁾ Pindolol is another representative antihypertensive beta-blocker with intrinsic sympathomimetic action. Since diltiazem possesses negative chronotropic action,²⁾ combined administration of diltiazem and pindolol appears to be reasonable. However, there are few investigations that concentrated on differences between diltiazem and nifedipine in hypertensive animals³⁾ and the combined effects of Ca an-

tagonists with pindolol.

The rat is the only hypertensive animal model in which acute and chronic antihypertensive effects of diltiazem were studied. On the other hand, the metabolic pathway of diltiazem in dogs is said to more closely resemble that in humans than in rats.⁴⁾ Thus, we studied the hypotensive actions of diltiazem and nifedipine, and the influence of pindolol in conscious renal hypertensive dogs (one kidney, one figure 8). In addition, consecutive administration of diltiazem was carried out in our model to study whether the tolerance, which was reported in the case of antihypertensive vasodilator hydralazine, develops with diltiazem.

MATERIALS AND METHODS

1. Experimental Preparation — Adult male beagle dogs, weighing between 10 and 15 kg, were used. Renal hypertension was induced by the method that has been previously described by Grollman.⁵⁾

* This work was presented in part at the 68th annual meeting of Japanese Pharmacological Society in Shizuoka, Japan, June 1983.

Dogs were anesthetized with pentobarbital Na (30 mg/kg, i.v.) and laparotomy was carried out at the left side under sterile conditions. The left kidney was compressed over the pole and the body with an umbilical tape. The incision was sutured and antibiotics (streptomycin sulfate and penicillin G) were injected intramuscularly at the lower extremities. The contralateral kidney was removed a week after the above mentioned treatment.

2. Blood Pressure and Heart Rate Measurements, and Electrocardiogram Recording — More than 2 months after the surgical procedures, a dog was anesthetized with pentobarbital Na (30 mg/kg, i.v.). A vinyl tubing (Extension tube[®]), which was filled with heparin Na solution (1000 units/ml), was inserted into the right or the left femoral artery. The other end of the tubing was guided under the skin and fixed at the back of the neck. The operation was performed under a sterile conditions.

Blood pressure was measured by telemetering with a pressure transducer (Century Technology, CP-01) connected to the arterial catheter and a transmitter (Nihon Kohden, ZB-653P) placed on the back of the dog. When the electrocardiogram was recorded, electrodes were attached to the skin and standard limb lead I was recorded by telemetering. Blood pressure and the electrocardiogram were simultaneously recorded on a recticorder (Nihon Kohden, WI-640G-S). Heart rate was determined by counting the pressure pulse for 15 s. Mean blood pressure was determined by adding $1/3$ of the difference between systolic and diastolic pressure to the diastolic pressure.

3. Experimental Procedure — **3-1. Single Oral Administration:** Effects of diltiazem and nifedipine were studied at doses of 1 and 4 mg/kg. Blood pressure and heart rate were measured in this experiment. Drugs were used in powder form, packed in capsules and administered orally after both blood pressure and heart rate were stabilized. Immediately after the administration, dogs were given 20 ml of water.

3-2. Coadministration with Pindolol: Thirty μ g/kg of pindolol were combined with 2 mg/kg of diltiazem or 2 mg/kg of nifedipine in this experiment. Combined drugs were packed in the same capsule. Administration of drugs were performed as mentioned previously. Blood pressure, heart rate and the PQ interval of the electrocar-

diogram were measured in this experiment.

3-3. Consecutive Administration: Before the start of the experiment, stabilities of blood pressure, heart rate and the PQ interval of the electrocardiogram were confirmed for 2 d. Diltiazem, 60 mg, was administered orally three times a day for 10 consecutive days. Since durability of the drug was thought to be important, two Herbesser tablets[®], which are release controlled form and packed in a capsule, were used in this experiment. Diltiazem was administered at 8:00, 13:00 and 18:00. Time courses of blood pressure, heart rate and the PQ interval were determined after the first administration on the first day as well as on the last day. Measurements were also performed just before the third administration each day, and 12 and 24 h after the last administration.

3-4. Plasma Level: The time course of plasma level of diltiazem after a single oral dose of 4 mg/kg was determined in 2 dogs. The time course of plasma level was also determined on the first day and the last day of consecutive administrations in 2 dogs. Four ml of the blood was sampled in an chilled tube from the implanted arterial catheter. Coagulation of the blood was inhibited by 4 mg of ethylenediaminetetraacetic acid (EDTA-Na). The blood was centrifuged (0–2 °C, $400 \times g$, 15 min) and the plasma was obtained. The plasma was frozen and stocked until assays were performed. The plasma level of diltiazem was determined by high performance liquid chromatography (HPLC) and ultraviolet (UV) detector (240 nm).

4. Statistical Methods — Data are expressed as means $\pm$ S.E.M. Statistical analysis was performed by paired *t*-test and when $p < 0.05$, results were considered to be significant.

RESULTS

1. Single Oral Administration

As shown in Fig. 1, blood pressure began to decrease soon after oral administration of diltiazem and a maximum response was observed during 30–60 min after administration. Four mg/kg of diltiazem decreased mean blood pressure about 20 mmHg and the decrease in blood pressure lasted for more than 5 h. Heart rate increased about 35 beats/min during the period 15 to 45 min after the administration and the rate decreased to the initial rate by 4 h.

Nifedipine, at a dose of 4 mg/kg, showed

FIG. 1. Time Courses of Effects of Diltiazem at a Dose of 4 mg/kg, p.o. on Systolic, Mean and Diastolic Blood Pressure and Heart Rate in Conscious Renal Hypertensive Dogs
Each point and vertical bar represent mean $\pm$ S.E.M. of 7 experiments.

FIG. 2. Time Courses of Effects of Nifedipine at a Dose of 4 mg/kg, p.o. on Systolic, Mean and Diastolic Blood Pressure and Heart Rate in Conscious Renal Hypertensive Dogs
Each point and vertical bar represent mean $\pm$ S.E.M. of 6 experiments.

almost the same hypotensive potency as that of diltiazem at the same dose (Fig. 2). However, the peak response was observed later than that in the case of diltiazem. Heart rate increased about 50 beats/min during the period 30–60 min after the administration. Hypotension and tachycardia lasted for more than 5 h (Fig. 2).

These effects of diltiazem and nifedipine were also observed at a smaller dose, 1 mg/kg (Figs. 3 and 4). The decrease in blood pressure was about 10 mmHg. Nifedipine-induced increase in heart rate was larger than that induced by diltiazem at this dose.

2. Coadministration with Pindolol

When diltiazem, 2 mg/kg, was administered alone, mean blood pressure decreased about 10 mmHg, heart rate increased about 25 beats/min and the PQ interval of the electrocardiogram was prolonged about 25 ms. On the other hand, pindolol, at a dose of 30 μ g/kg, decreased mean blood pressure about 15 mmHg, increased heart

rate about 40 beats/min and shortened the PQ interval about 10 ms. When both drugs were combined, mean blood pressure decreased additively (about 20 mmHg). However, an increase in heart rate was greater than that caused by diltiazem alone and smaller than that caused by pindolol alone. Diltiazem-induced prolongation of the PQ interval was not affected (Fig. 5).

When nifedipine, 2 mg/kg, was administered individually, mean blood pressure decreased about 10 mmHg and the heart rate increased about 50 beats/min. The combination of nifedipine with pindolol not only enhanced hypotension but tachycardia also became more pronounced (Fig. 6).

3. Consecutive Administration

Figure 7 shows the time courses in mean blood pressure, heart rate and the PQ interval of the electrocardiogram on the first day and on the last day of consecutive administration. On the first day, diltiazem decreased mean blood

FIG. 3. Time Courses of Effects of Diltiazem at a Dose of 1 mg/kg, p.o. on Systolic, Mean and Diastolic Blood Pressure and Heart Rate in Conscious Renal Hypertensive Dogs

Each point and vertical bar represent mean $\pm$ S.E.M. of 5 experiments.

FIG. 4. Time Courses of Effects of Nifedipine at a Dose of 1 mg/kg, p.o. on Systolic, Mean and Diastolic Blood Pressure and Heart Rate in Conscious Renal Hypertensive Dogs

Each point and vertical bar represent mean $\pm$ S.E.M. of 5 experiments.

FIG. 5. Combined Effects of Diltiazem and Pindolol on Mean Blood Pressure, Heart Rate and PQ Interval of Electrocardiogram in Conscious Renal Hypertensive Dogs

○, diltiazem 2 mg/kg, p.o.; △, pindolol 30 μ g/kg, p.o.; ■, diltiazem 2 mg/kg, p.o. and pindolol 30 μ g/kg, p.o. a) and b) represent significant differences between diltiazem and combination at $p < 0.05$ and $p < 0.01$, respectively. c) and d) represent significant differences between pindolol and combination at $p < 0.05$ and $p < 0.01$, respectively. Each point and vertical bar give mean $\pm$ S.E.M. of 5 experiments.

pressure about 20 mmHg, increased heart rate about 20 beats/min and prolonged PQ interval about 55 ms. Although the effects on mean blood pressure and the PQ interval lasted for more than 5 h, the increase in heart rate was followed by a decrease by 3 h or later.

On the last day, preadministration values of mean blood pressure and heart rate were slightly lower than those on the first day. The PQ interval was almost the same as that on the first day. Maximum responses were similar to those observed on the first day. Duration of hypotensive action was similar to that on the first day, whereas durations of the increase in heart rate and a prolongation of the PQ interval were shorter than those on the first day. Heart rate apparently decreased by 3 h or later.

Figure 8 shows the parameters measured just

FIG. 6. Combined Effects of Nifedipine and Pindolol on Mean Blood Pressure, Heart Rate and PQ interval of Electrocardiogram in Conscious Renal Hypertensive Dogs

○, nifedipine 2 mg/kg, p.o.; △, pindolol 30 µg/kg, p.o.; ■, nifedipine 2 mg/kg, p.o. and pindolol 30 µg/kg, p.o. a) and b) represent significant differences between nifedipine and combination at $p < 0.05$ and $p < 0.01$, respectively. c) and d) represent significant differences between pindolol and combination at $p < 0.05$ and $p < 0.01$, respectively. Each point and vertical bar give mean $\pm$ S.E.M. of 5 experiments.

before the third administration of each day and 12 and 24 h after the last administration. On the first day, mean blood pressure was lower than control level by about 10 mmHg. Thereafter, it decreased further and reached about 15 mmHg below the control level on the third day. It remained at this level until the last day and was still significantly lower than the control level 24 h after the last administration. The heart rate showed slightly higher level on the first day (about 10 beats/min) but then gradually decreased. On the fifth day or later, it was about 5 beats/min lower than the control level. It recovered to the control level 24 h after the last administration. The PQ interval was prolonged about 35 ms initially but the prolongation began

FIG. 7. Time Courses of Effects of Diltiazem at a Dose of 60 mg, p.o. on Mean Blood Pressure, Heart Rate and PQ interval of Electrocardiogram in Conscious Renal Hypertensive Dogs on the Starting (○) and the Last (●) Day of Consecutive Administration Study for 10 d

Each point and vertical bar represent mean $\pm$ S.E.M. of 4 experiments.

to diminish on the fourth day or later. The PQ interval was prolonged only 5 ms on the last day. It was shortened 12 and 24 h after the last administration.

4. Plasma Level

The time course of the plasma level after a single oral administration of diltiazem (4 mg/kg) is shown in Fig. 9A. Time courses of the plasma level after 60 mg of diltiazem on the first day and on the last day of consecutive administration are also shown in Fig. 9B.

As shown in Fig. 9A, peak levels were observed 15 and 60 min after the single oral administration of diltiazem; they were about 4 and 7×10^{-7} g/ml. Thereafter, they gradually decreased to about $0.5 - 1 \times 10^{-7}$ g/ml 5 h after the administration.

On the other hand, peak levels were observed at 1 and 2 h after the administration both on the

FIG. 8. Antihypertensive Action of Diltiazem in Conscious Renal Hypertensive Dogs

Diltiazem at a dose of 60 mg, p.o. was administered 3 times a day and each parameter was measured just before the third administration of the day. Each point and vertical bar represent mean $\pm$ S.E.M. of 4 experiments. a, b) Significantly different from the values on the day before the start of the consecutive administration (-1 day) at $p < 0.05$ and $p < 0.01$, respectively.

first day and on the last day in the experiment of consecutive administration (Fig. 9B). The time of peak level was constant in the individual dog. Peak levels on the starting day was $2.5-3 \times 10^{-7}$ g/ml but were $4-4.5 \times 10^{-7}$ g/ml on the last day. Five h after the administration, the level was $1-2 \times 10^{-7}$ g/ml in every case. Plasma level was almost zero at 24 h after the last administration.

DISCUSSION

It was reported that the peak plasma level was $1-5 \times 10^{-7}$ g/ml after oral administration of diltiazem at doses of 120–180 mg in humans.⁶⁾ These doses correspond to 2–3 mg/kg, on the assumption that the body weight is 60 kg. In this study, the peak plasma level of diltiazem at a dose of 4 mg/kg was found to be

FIG. 9. Time Courses of Plasma Levels of Diltiazem

(A) A dose of 4 mg/kg, p.o. in 2 conscious renal hypertensive dogs.

(B) A dose of 60 mg, p.o. in renal hypertensive dogs on the starting ($\circ$) and the last ($\bullet$) day of consecutive administration study for 10 d. B-1 and B-2 gives individual data of 2 dogs.

$4-7 \times 10^{-7}$ g/ml in renal hypertensive dogs. The dose, 4 mg/kg, which is close to a clinical dose, caused apparent hypotension in renal hypertensive dogs. Therefore, the relationship between the dose, the plasma level and the hypotensive action of diltiazem in dogs resembles that in humans. The main metabolite of diltiazem in dogs is also similar to that in humans.⁴⁾

Although the control group was not studied in our experiment, potencies and durabilities of responses to diltiazem and nifedipine were dose-dependent. Their hypotensive actions were nearly identical at the same doses and their hypotensive responses lasted for more than 5 h. Diltiazem-induced tachycardia, which was ascribed to baroreflex due to the hypotension, recovered within 4 h. However, nifedipine developed a greater and longer increase in heart rate than that caused by diltiazem.

It was reported that the negative chronotropic action of diltiazem was relatively stronger than

that of nifedipine.²⁾ Moreover, Nakaya *et al.* suggested suppressive action of diltiazem on reflex tachycardia due to hypotension.¹⁾ On the other hand, Heesch *et al.* reported excitatory action of nifedipine on the carotid sinus baroreceptors in isolated preparations of dogs⁷⁾ and Millard *et al.* reported suppressive action of nifedipine on reflex bradycardia mediated by parasympathetic nervous system.⁸⁾ These properties may cause the difference between the effects of diltiazem and nifedipine on heart rate.

Pindolol caused marked hypotension and tachycardia in our experiment. Since the control value of heart rate was as low as 76 beats/min, sympathetic tone of dogs should be low. Thus pindolol could cause tachycardia by its intrinsic sympathomimetic action. Reflex tachycardia due to a decrease in blood pressure should also contribute to the pindolol-induced increase in heart rate.

When pindolol was administered in combination with diltiazem, an increase in heart rate was smaller than that caused by an individual dose of pindolol. However, pindolol-induced tachycardia was enhanced in a combination experiment with nifedipine. This result was probably due to the negative chronotropic action of diltiazem and also to the non-competitive inhibitory action of diltiazem on tachycardia induced by beta-adrenergic stimulation.⁹⁾ Effects of diltiazem and nifedipine on baroreflex also can not be ruled out.

Hypotensive action of diltiazem did not linearly correlate with its plasma level in the experiment of consecutive administration: the plasma level was almost zero 24 h after the last administration. Even at this time, blood pressure was still lower than the control level. This carry-over effect of diltiazem after consecutive administration was also observed in another experimental hypertensive model, spontaneously hypertensive rats (SHR).¹⁰⁾ These phenomena may be explained by assuming that diltiazem possesses properties which cause functional changes in blood vessels and/or changes in body fluid distribution. Blood pressure and heart rate were measured 48 h after the last administration in 3 cases, no withdrawal effects were observed.

Heart rate increased initially and decreased later. Prolongation of the PQ interval began to diminish on the fourth day or later. It is apparent from the time courses on the first day and on the

last day that these changes were the results of more rapid recoveries in tachycardia and prolongation of the PQ interval. It remains to be explained why these rapid recoveries occurred.

In summary, diltiazem caused hypotension in renal hypertensive dogs at doses which were comparable to clinical doses. The same doses of diltiazem and nifedipine caused similar hypotension, but diltiazem-induced tachycardia was less than that caused by nifedipine. While hypotensive actions of diltiazem and nifedipine increased by combination with pindolol, pindolol-induced tachycardia was suppressed with diltiazem, but was more pronounced with nifedipine. When diltiazem was consecutively administered, the hypotensive action was not affected but the reflex tachycardia lessened. Prolongation of the PQ interval, which is one of the side effects of diltiazem, also diminished. No withdrawal effects were observed. It is suggested that diltiazem is an effective drug for long term treatment of hypertension.

REFERENCES

- 1) H. Nakaya, A. Schwartz and R. W. Millard: Reflex chronotropic and inotropic effects of calcium channel-blocking agents in conscious dogs. Diltiazem, verapamil and nifedipine compared, *Circ. Res.*, **52**, 302–311 (1983).
- 2) H. Ono, N. Himori and N. Taira: Chronotropic effects of coronary vasodilators as assessed in the isolated, blood-perfused sino-atrial node preparation of the dog, *Tohoku J. Exp. Med.*, **121**, 383–390 (1977).
- 3) H. Narita, N. Nagao, M. Otsuka, H. Yabana and I. Yamaguchi: Hypotensive and diuretic actions of diltiazem in SHR, *Jpn. J. Pharmacol.*, **32** (suppl), 60p (1982).
- 4) J. Sugihara, Y. Sugawara, H. Ando, S. Harigaya, A. Etoh and K. Kohno: Studies on the metabolism of diltiazem in man, *J. Pharmacobio-Dyn.*, **7**, 24–32 (1984).
- 5) A. Grollman: A simplified procedure for inducing chronic renal hypertension in the mammal, *Proc. Soc. Exp. Biol. Med.*, **57**, 102–104 (1944).
- 6) P. L. Morselli, M. Rovei, M. Mitchard, A. Durand, R. Gomeri and J. Larribaud: Pharmacokinetics and metabolism of diltiazem in man (observations on healthy volunteers and angina pectoris patients), "New Drug Therapy with a Calcium Antagonist," ed. by R. J. Bing, Excerpta Medica, Amsterdam, 1979, pp. 152–168.
- 7) C. M. Heesch, B. M. Miller, M. D. Thames and F. M. Abboud: Effects of calcium channel blockers on isolated carotid baroreceptors and baroreflex, *Am. J. Physiol.*, **245**, H653–661 (1983).
- 8) R. W. Millard, D. A. Lathrop, G. Grupp, M. Ashraf, I. L. Grupp and A. Schwartz: Differential cardiovascular effects of calcium channel blocking agents: Potential

- mechanisms, *Am. J. Cardiol.*, **49**, 499–506 (1982).
- 9) K. Yamada, T. Shimamura and H. Nakajima : Studies on a new 1,5-benzothiazepine derivative (CRD-401). V. Antiarrhythmic actions, *Jpn. J. Pharmacol.*, **23**, 321–328 (1973).
- 10) M. Sato, S. Murata, H. Narita, M. Tomita, K. Yamashita and I. Yamaguchi : Hypotensive effects of diltiazem hydrochloride in the normotensive, spontaneously hypertensive and renal hypertensive rats, *Folia Pharmacol. Jpn.*, **75**, 99–106 (1979).