Cardiovascular Effects of YM430, a 1,4-Dihydropyridine Derivative with β -Adrenoceptor Blocking Activity, in Dogs and Rats

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We evaluated the cardiovascular effects of YM430, a novel 1,4-dihydropyridine derivative with β -adrenoceptor blocking activity, in dogs and rats. In anesthetized dogs, YM430 (0.01–0.3 mg/kg, i.v.) dose-dependently decreased mean blood pressure, total peripheral resistance and double product without increasing the heart rate. YM430 (0.01–0.3 mg/kg, i.v.) increased coronary artery as well as vertebral artery blood flow, whereas its effects on carotid, mesenteric, femoral and renal blood flow were small. At the same dose range as that which induced vasodilation effects, YM430 had little effect on the max. dp/dt or PQ-interval. In conscious dogs, YM430 (0.1–1 mg/kg, i.v.) produced dose-dependent hypotension with tachycardia. In conscious rats, oral dosing of YM430 (100 mg/kg *p.o.*) produced a long-lasting hypotensive effect with slight tachycardia. YM430 also inhibited isoproterenol (0.1 μ g/kg i.v.)-induced tachycardia. These two effects of YM430 may be attributable to its calcium entry blocking and β_1 -adrenoceptor blocking activity, respectively. The time course of the hypotensive (calcium entry blocking) effect of YM430 after oral dosing was very similar to that of its inhibition of isoproterenol-induced tachycardia (β_1 -adrenoceptor blocking effect). These results indicate that the ratio of the two activities (calcium entry blocking and β_1 -adrenoceptor blocking) of YM430 is constant after oral administration. In conclusion, YM430 could be both an antianginal and antihypertensive agent, because of its dual activities.

Key words YM430; hypotension; antiangina; calcium antagonist; β -blocker; cardiovascular effect

While both calcium entry blocking agents and β -adrenoceptor blocking agents have been successfully used to treat cardiovascular disease such as hypertension and angina pectoris, each class of drug has inherent properties that may prove undesirable to the individual patient.^{2–6)} One problem with some calcium entry blocking agents, especially 1,4-dihydropyridines, is that they induce a reflex increase in sympathetic tone. In animals and humans, the blood pressure (BP) lowering effect of calcium entry blocking agents in this class results in the baroreceptor-mediated reflex sympathetic nerve stimulation, which may well reduce the hypotensive effect by increasing the heart rate (HR).^{7,8)} On the other hand, β -adrenoceptor blocking agents have been associated with disturbances in peripheral circulation and depression cardiac function.⁴⁾ In addition, the blockade of coronary artery β -adrenoceptors might worsen vasospasm by converting the effect of a sympathetic stimulus into a purely α -adrenergic vasoconstrictor response.⁹⁾ Therefore, the combined use of a calcium entry blocking agent and a β -adrenoceptor blocking agent has recently been recognized as an alternative treatment for hypertension and angina pectoris. Indeed, recent studies demonstrating that a combined treatment of calcium entry blocking agent and β -adrenoceptor blocking agent has therapeutic advantages over either agent alone in the treatment of hypertension and angina have supported this rationale.^{10–12)} YM430 (Fig. 1) is a new 1,4-dihydropyridine derivative that possesses both calcium entry blocking and β -adrenoceptor activities in one molecule.^{13,14)} According to the rational outline above, a drug possessing both activities would appear to have a desirable therapeutic profile in the treatment of hypertension and angina pectoris. Thus, in the present study we examined the cardiovascular effects of YM430 in dogs and rats to evaluate the potential clinical use of

this compound as an antihypertensive and antianginal agent.

MATERIALS AND METHODS

Experimental Procedures All experiments were approved by the Animal Ethics Committee of Yamanouchi Pharmaceutical Co., Ltd.

Cardiovascular Effects Experiments were performed on 18 mongrel dogs of either sex weighing 8–17 kg. Animals were anesthetized with 30 mg/kg sodium pentobarbital i.v.; a constant level of anesthesia was then maintained by i.v. infusion of sodium pentobarbital at a rate of 3–5 mg/kg/h with an infusion pump (Harvard Apparatus, model 944) through the forearm vein. After the animals were intubated, artificial respiration was performed with a respiration pump (SN-480-4, Shinano Seisakusho, Tokyo, Japan), with room air administered at 18 strokes/min (20 ml/kg tidal volume). The chest was opened at the fourth intercostal space. An electromagnetic flow probe was placed around the left circumflex coronary artery (LCX) and descending aorta. Coronary blood flow (Cor.BF) and cardiac output (CO) were measured by an electromagnetic flowmeter (MFV-3100, Nihon Koden,

Fig. 1. Chemical Structure of YM430, [4-(((*S*)-2-Hydroxy-3-phenoxy-propyl)amino)butyl methyl 2,6-dimethyl-((*S*)-4-(*m*-nitrophenyl))-1,4-dihydropyridine-3,5-dicarboxylate]

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Tokyo, Japan), through a electromagnetic flow probe. Systemic blood pressure in the femoral artery was measured with a pressure transducer (AP-400T, Nihon Kodens Tokyo, Japan), and heart rate was measured using a cardiometer (AT-600G, Nihon Kodens) triggered by the pulse of arterial BP. A polyvinyl catheter for the drug injection was implanted into the femoral vein. Double product (DP), left ventricular work (LVW), and total peripheral resistance (TPR) were calculated: DP (mmHg · beats/min) = SBP (mmHg) × HR (beats/min), LVW (kg · m/min) = $0.0136 \times \text{MBP (mean blood pressure, mmHg)} \times \text{CO (l/min)}$, TPR (dyn · s/cm⁵) = $80 \times \text{MBP (mmHg)} / \text{CO (l/min)}$. Changes in all parameters were recorded on an ink-writing oscillograph (RM-6000, Nihon Kodens). All test drugs were administered i.v.

Effects on Arterial Blood Flow Experiments were performed on 10 mongrel dogs of either sex weighing 8–13 kg. The anesthetic and surgical procedures were the same as those described above. An electromagnetic flow probe was placed around either the right vertebral, left carotid, superior mesenteric, left renal or right femoral artery in closed-chest dogs and around LCX in open-chest dogs. Vertebral (VBF), carotid (Car.BF), coronary artery (Cor.BF), mesenteric (MBF), renal (RBF) or femoral (FBF) artery blood flow were measured with an electromagnetic flowmeter through each electromagnetic flow probe. Vascular resistance (VR) was calculated as: VR (dyn · s/cm⁵) = $80 \times \text{MBP (mmHg)} / \text{blood flow (ml/min)}$. All parameters were displayed on an ink-writing oscillograph. The test drug was administered i.v.

Effects on the Atrioventricular (AV) Conduction System Experiments were performed on 4 mongrel dogs of either sex weighing 9–12 kg. The anesthetic and surgical procedures and methods for measuring BP and HR were the same as those described above. A catheter tip manometer (PC-350A, Millar Instruments, Houston, TX, U.S.A.) was inserted into the left ventricle through the left carotid artery for measurement of left ventricular (LV) pressure. LV dp/dt max. was obtained with an electro-differentiator (EQ-600G, Nihon Kohden). ECG (electrocardiogram, lead II) was measured, and PQ-interval was used as an index of AV conduction time. The test drug was administered i.v.

Hypotensive Effect in Conscious Dogs Experiments were performed on 7 beagle dogs of either sex weighing 10–14 kg. Under GOF anesthesia (N₂O:O₂ = 2:1, halothane 1%), a polyvinyl catheter for recording arterial BP was surgically implanted in the abdominal aorta through a muscular branch of the femoral artery. Animals were used for experiments after a 1-week recovery period. During the recovery period, all animals were housed in individual experimental cages and given a dry-type dog meal and water *ad libitum*. BP and HR were measured continuously while animals were immobilized in a comfortable hammock. ECG (lead II) was also recorded. The test drug was administered i.v.

Time Course of Hypotensive and β_1 -Adrenoceptor Blocking Effects in Rats Experiments were done using 8 male Wistar rats weighing 300 to 330 g. After animals were anesthetized with ether, polyethylene catheters (Intramedic PE-50, Clay Adams, Parsippany, NJ, U.S.A.)

for measuring arterial BP and injecting isoproterenol were implanted into the carotid artery and into the jugular vein, respectively. At 3 d after operation, arterial BP and HR were measured under unrestrained conditions. During the recovery period, all animals were housed in individual experimental cages and given a dry-type rat meal and water *ad libitum*. Arterial BP was measured by a pressure transducer (AP-200T, Nihon Kodens, Tokyo, Japan) connected to an implanted catheter. HR was measured with a cardiometer (AT-600G, Nihon Kodens) triggered by the arterial BP pulse wave. YM430 (100 mg/kg *p.o.*) and nifedipine (10 mg/kg *p.o.*) plus atenolol (30 mg/kg *p.o.*) were suspended in a 0.5% methylcellulose solution and given by oral gavage at 5 ml/kg. To assess β_1 -adrenoceptor blocking activity, isoproterenol (0.1 $\mu\text{g/kg}$ i.v.) was injected immediately after measurement of BP and the increase in HR was measured.

Materials YM430 was prepared by Yamanouchi Pharmaceutical Co., Ltd. Nifedipine, atenolol and isoproterenol hydrochloride (Sigma Chemical, St. Louis, MO, U.S.A.) were obtained commercially. YM430 was dissolved in distilled water containing 1 drop of 1 N HCl and 20% ethanol, and nifedipine was dissolved in distilled water containing 20% ethanol. Atenolol was dissolved in a 0.9% saline solution. Isoproterenol hydrochloride was dissolved in 0.9% saline solution containing 0.01% ascorbic acid.

Statistical Analysis The data are expressed as the mean $\pm$ S.E.M. Comparison among the groups was performed using Student's *t*-test or one-way analysis of variance (one-way ANOVA). *p* values of less than 0.05 were considered to be statistically significant.

RESULTS

Cardiovascular Effects Baseline values were not significantly different between each group (Student's *t*-test) (Table 1). In anesthetized dogs, YM430 at doses of 0.01–0.3 mg/kg i.v. decreased MBP, TPR and DP, and increased Cor.BF in a dose-dependent manner (Fig. 2) without a large change in HR or LVW. Nifedipine at 0.0003–0.01 mg/kg i.v. resembled YM430 in its effect on all parameters except HR, which increased. Hypotensive and vasodilating activities of YM430 were about 30 to 100 times less potent than that of nifedipine, but these effects of YM430 (0.3 mg/kg i.v.) lasted longer than those of nifedipine (0.003 mg/kg i.v.), and the action of YM430 returned to base line about 120 min after administration (Fig. 3).

Effects on Arterial Blood Flow In anesthetized dogs,

Table 1. Baseline Values of Cardiovascular Parameters before i.v. Administration of YM430 and Nifedipine in Anesthetized Dogs

Parameters	YM430	Nifedipine
HR (beats/min)	159 $\pm$ 10	173 $\pm$ 13
MBP (mmHg)	112 $\pm$ 8	121 $\pm$ 3
TPR (dyn · s/cm ⁵)	8037 $\pm$ 1852	7377 $\pm$ 840
Cor.BF (ml/min)	56.0 $\pm$ 8.2	39.2 $\pm$ 3.5
DP (mmHg · beats · 10 ⁻³ /min)	22.1 $\pm$ 2.5	25.3 $\pm$ 1.7
LVW (kg · m/min)	2.0 $\pm$ 0.5	2.3 $\pm$ 0.3

Values are the mean $\pm$ S.E.M. of 4–5 dogs.

Fig. 2. Effect of Intravenous Administration of YM430 and Nifedipine on HR, MBP, TPR, Cor.BF, DP and LVW in Anesthetized Dogs
○, YM430; ●, nifedipine. Each point represents the mean $\pm$ S.E.M. of 4–5 dogs.

YM430 at doses of 0.01–0.3 mg/kg i.v. increased Cor.BF as well as vertebral blood flow in a dose-dependent manner. However, changes in carotid, mesenteric, renal and femoral blood flow were small (Fig. 4). In contrast, all vascular resistance except renal vascular resistance decreased. Compared according to the decrease (%) in vascular resistance at 0.3 mg/kg i.v., the degree of vascular reactivity to YM430 was ranked as follows: vertebral > coronary > carotid = femoral > mesenteric > renal artery (Table 2).

Effects on the AV Conduction System YM430 at doses of 0.01–0.3 mg/kg i.v. decreased MBP, with a slight change in HR and PQ-interval and max. dp/dt in anesthetized dogs (Fig. 5).

Hypotensive Effect in Conscious Dogs Baseline values were not significantly different among groups (one-way ANOVA) (Table 3). In conscious dogs, the i.v. administration of YM430 at doses of 0.1–1 mg/kg produced a dose-dependent hypotensive effect with an increase in HR but no change in PQ-interval. At 1 mg/kg i.v. of YM430, the maximal changes of MBP and HR were -36.5 ± 2.7 (mmHg) and 63.0 ± 8.2 (beats/min), respectively. The hypotensive effect of YM430 reached its maximum within 15 min after dosing and lasted 3–4 h (Fig. 6).

Time Course of Hypotensive and β_1 -Adrenoceptor Blocking Effects in Rats Baseline values were not significantly

different between groups (Student's *t*-test) (Table 4). The effects of YM430 (100 mg/kg *p.o.*) and the combination of nifedipine (10 mg/kg *p.o.*) plus atenolol (30 mg/kg *p.o.*) on BP and HR are shown in Fig. 7. We confirm that nifedipine at 10 mg/kg *p.o.* and atenolol at 30 mg/kg *p.o.* are equipotent to YM430 at 100 mg/kg *p.o.* in decreasing MBP and inducing β -adrenoceptor blocking effect, respectively (data not shown). YM430 produced a hypotensive effect (-31.5 ± 5.0 mmHg) with an increase in HR (46.7 ± 12.4 beats/min), but this change in HR was not significant. In contrast, nifedipine produced a bigger and significant increase in HR (110.7 ± 15.6 beats/min). Its hypotensive effect reached the maximum about 2 h after dosing and lasted more than 8 h. YM430 inhibited isoproterenol-induced tachycardia, and this effect also reached a maximum at about 2 h and lasted over 8 h after dosing. The time courses of hypotensive effect and inhibition of isoproterenol-induced tachycardia were very similar, and the response ratio was constant. The combination of nifedipine plus atenolol also produced hypotensive effects without a change in HR. However, the time course of this combination on hypotension was different from that of its inhibition of isoproterenol-induced tachycardia.

Fig. 3. Time Course Changes in HR, MBP, TPR, Cor.BF, DP and LVW after Intravenous Administration in Anesthetized Dogs
○, YM430 0.3 mg/kg i.v.; ●, nifedipine 0.003 mg/kg i.v. Each point represents the mean $\pm$ S.E.M. of 4–5 dogs.

DISCUSSION

In this study, we evaluated the cardiovascular actions of YM430 using dogs and rats. In anesthetized dogs, YM430 resembled nifedipine in increasing Cor. BF and in decreasing MBP, TPR and DP, but differed from nifedipine in decreasing HR. Thus, in contrast to nifedipine, YM430 appears to decrease MBP effectively without causing a reflex increase in HR. Calcium entry blocking agents of the 1,4-dihydropyridine type are believed to cause a reflex increase in sympathetic tone in response to induced systemic vasodilation.^{7,8)} Indeed, after i.v. administration of these compounds, reflex tachycardia has been observed; this tachycardia was abolished by either β -adrenoceptor blocking agents or denervation of the baroreceptor afferent nerves.¹⁵⁾ In previous studies, it was demonstrated that YM430 possessed β -adrenoceptor blocking activity as well as calcium entry blocking activity in one molecule, and YM430 showed about a 100-fold greater selectivity for β_1 - than for β_2 -adrenoceptor in *in vitro* study.^{13,14)} The fact that YM430 did not produce reflex tachycardia could be related to the β_1 -adrenoceptor blocking activity of this drug, and not be attributable to the inhibition of norepinephrine release by β_2 -adrenoceptor blocking effect. Furthermore, the duration of the vasodilating effect of YM430 is longer than that of nifedipine at doses which produced a similar degree of hypotension. This long duration of effect of YM430 after

i.v. administration might be one more advantage over nifedipine in clinical use.

Our experiments in anesthetized dogs demonstrated that the increasing effect of YM430 on arterial blood flow was greater in vertebral and coronary vessels than in carotid, mesenteric, renal and femoral vessels, and all VR except in renal artery were lowered by YM430. These results show that YM430 produces a selective and direct increase in VBF and Cor. BF. YM430 also produced a decrease in DP in anesthetized dogs. Thus, YM430 increases the myocardial oxygen supply without increasing oxygen demand, and clearly improves the balance between myocardial oxygen supply and demand. Therefore, YM430 probably also possesses favorable properties as a remedy for angina pectoris.

The combination of calcium entry blocking agents with β -adrenoceptor blocking agents has recently been proposed for the treatment of systemic hypertension and angina pectoris.^{10,11)} Since calcium entry blocking agents inhibit transmembrane calcium influx in cardiac muscle as well as vascular smooth muscle, a cardiodepressant effect is often observed. In general, the dihydropyridine calcium entry blocking agents are vascular selective, while the non-dihydropyridines, such as verapamil and diltiazem, have more prominent effects on the calcium channels in the sinoatrial node (decreased HR) and myocardium (negative inotropic effect). On the other hand, β -adrenoceptor blocker such as propranolol, suppressed left

Fig. 4. Effect of YM430 on Blood Flow and Vascular Resistance of the Vertebral (○), Coronary (●), Carotid (△), Renal (□), Mesenteric (■) and Femoral (▲) Arteries in Anesthetized Dogs

Each point represents the mean $\pm$ S.E.M. of 5 dogs.

Table 2. Decreasing Effects of YM430 on Vascular Resistance in Anesthetized Dogs

Arteries	Vascular resistance decrease (%) at 0.3 mg/kg i.v. of YM430
Vertebral	-74.0 ± 3.6
Coronary	-65.5 ± 3.2
Carotid	-51.2 ± 3.7
Femoral	-47.8 ± 6.0
Mesenteric	-39.4 ± 6.6
Renal	-1.30 ± 2.8

Values are the mean $\pm$ S.E.M. of 5 dogs.

ventricular contractility, sinus node activity and AV-conduction in anesthetized dogs.¹⁶⁾ Therefore, in combined treatment with a calcium entry blocking agent and β -adrenoceptor blocking agent, its effect on the AV-conduction system is a crucial factor. In our study, YM430 had almost no effect on HR, PQ-interval or max. dp/dt at the doses at which it produced hypotension. These data suggest that no cardiodepressant effect of YM430 was induced by its possession of both calcium entry blocking and β -adrenoceptor blocking activities in one molecule.

In conscious dogs, i.v. administration of YM430 produced a dose-dependent hypotensive effect with an

Fig. 5. Effect of Intravenous Administration of YM430 on HR, MBP, PQ-Interval and Max. dp/dt in Anesthetized Dogs

Each point represents the mean $\pm$ S.E.M. of 4 dogs.

Table 3. Baseline Values of HR, PQ-Interval and MBP before i.v. Administrations of YM430 in Conscious Dogs

Parameters	YM430 (mg/kg i.v.)		
	0.1	0.3	1.0
HR (beats/min)	107 ± 8	110 ± 7	92 ± 5
PQ-Interval (ms)	97 ± 6	101 ± 2	96 ± 4
MBP (mmHg)	120 ± 9	118 ± 6	103 ± 7

Values are the mean $\pm$ S.E.M. of 4–6 dogs.

Fig. 6. Effects of Intravenous Administration of YM430 on HR, PQ-Interval, and MBP in Conscious Dogs

○, 0.1 mg/kg i.v.; ●, 0.3 mg/kg i.v.; △, 1.0 mg/kg i.v. Each point represents the mean $\pm$ S.E.M. of 4-6 dogs.

Table 4. Baseline Values of HR, MBP and ISO-Induced Tachycardia before Oral Administration of YM430 and of the Combination of Nifedipine and Atenolol in Conscious Rats

Parameters	YM430	Nifedipine + atenolol
HR (beats/min)	388 $\pm$ 8	370 $\pm$ 17
MBP (mmHg)	105 $\pm$ 13	80 $\pm$ 3
ISO-Induced tachycardia (beats/min)	108 $\pm$ 20	121 $\pm$ 13

Values are the mean $\pm$ S.E.M. of 4 rats.

increase in HR. However, it is known that i.v. administration of nifedipine produces a short-lasting hypotensive effect with a marked increase in HR and decrease in the PQ-interval.¹⁷⁾ Compared at the equipotent dose which caused a decrease in MBP, the increase in HR by YM430 (1 mg/kg i.v.; 63.0 $\pm$ 8.2 beats/min) is significantly smaller

Fig. 7. Time Course Change of YM430 and the Combination of Nifedipine + Atenolol on HR, MBP and Inhibition of Isoproterenol (ISO)-Induced Tachycardia in Conscious Rats

Ratio was calculated as: (inhibition% of ISO-induced increase in heart rate)/(% change in MBP). ○: YM430 100 mg/kg p.o.; ●: nifedipine 10 mg/kg p.o. + atenolol 30 mg/kg p.o. Each point represents the mean $\pm$ S.E.M. of 4 rats.

than that by nifedipine (0.1 mg/kg i.v.; 101.0 $\pm$ 7.4 beats/min¹⁷⁾). These results suggest that YM430 actually produced calcium entry blocking and β_1 -adrenoceptor blocking effects in conscious dogs when administered intravenously, and may have advantages over nifedipine in the treatment of hypertension.

The next series of experiments assessed the time course of the hypotensive and β -adrenoceptor blocking effects of YM430. Similar studies^{18,19)} have reported that, in use in the treatment of hypertension, β -adrenoceptor blocking agents do not induce an acute decrease in BP. We did not, therefore, anticipate that the β -adrenoceptor blockade of

YM430 would contribute appreciably to its immediate hypotensive effect in rats, and propose instead that this acute activity of YM430 was solely the result of its calcium entry blocking effect. Both activities reached maximum at about 2 h after oral dosing and lasted more than 8 h. In contrast, the time courses of these activities in combined administration (nifedipine and atenolol) were different. Indeed, the finding that, unlike the case with combination treatment, the response ratio (inhibition of isoproterenol-induced tachycardia/hypotension) of YM430 did not change suggests that these activities of YM430 are maintained in a constant ratio after oral administration.

In the treatment of angina pectoris and hypertension, great care is needed to establish the correct dosage and ratio of calcium entry blocking agents and β -adrenoceptor blocking agents.³⁾ However, the flexibility of varying the ratio of calcium entry blocking activity to β -adrenoceptor blocking activity is lost on the delivery of one compound with both activities. The therapeutic value of YM430, therefore, needs to be studied in many kinds of experimental models. The present study at least indicates that the favorable ratio for the two activities of YM430 was constant after oral dosing.

In conclusion, this study shows that YM430 actually behaves as a hybrid compound combining calcium entry blocking and β -adrenoceptor blocking activities in dogs and rats. In addition, it is suggested that YM430 could be a long-lasting antianginal and antihypertensive agent in clinical use.

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