Cardiovascular Pharmacology of RS-1893, an Orally Active Cardiotonic Agent with Arterial and Venous Vasodilator Actions

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Abstract—RS-1893, 2-[2-chloro-4-(2,3,4,5-tetrahydro-3-oxo-6-pyridazinyl)]-phenoxy-N-(2-morpholinoethyl)-acetamide, is a newly synthesized compound whose structure is different from that of cardiac glycosides and beta-stimulants. The in vitro cardiotonic action of RS-1893 was about 3 times more potent than that of milrinone. This action is most likely due to inhibition of phosphodiesterase-III, as has been suggested for many other cardiotonic agents. In pentobarbital anesthetized dogs, RS-1893 (1-30 μg/kg, i.v.) produced dose dependent increases in left ventricular dP/dtmax and cardiac output and caused decreases in blood pressure and total peripheral resistance with a relatively small increase in heart rate. Central venous pressure decreased markedly, suggesting venous vasodilation. The in vivo cardiotonic action of RS-1893 was 3 times more potent than that of milrinone and was not affected in the presence of a large dose of propranolol. Oral administration of RS-1893 (0.03 and 0.1 mg/kg) also produced a dose-related increase in cardiac contractility in conscious beagles. The increase in LVdP/dtmax reached a maximum in 1–3 hr after administration and lasted for more than 8 hr. Thus, RS-1893 appeared to be an orally active cardiotonic agent with vasodilator properties, probably acting on both arterioles and veins.

Numerous efforts have been made to develop orally active cardiotonics which are expected to replace cardiac glycosides and catecholamines in the management of congestive heart failure (1-6). However, it is still controversial whether these oral cardiotonics improve the prognosis of patients (7).

Another approach to the treatment of heart failure is the use of arterial vasodilators such as prazosin (8) and captopril (9). They reduce the afterload to the heart and increase tissue perfusion. More recently, venous vasodilators such as nitroglycerin and isosorbide dinitrate (ISDN) have been successfully used in patients with congestive heart failure (10, 11). Nitrates reduce preload to the heart and lower capillary pressure, thus diminishing edema (12, 13).

These facts led us to the notion that combination in one molecule of inotropic and arterial and venous vasodilator properties may provide hemodynamic benefit in patients with congestive heart failure. We have synthesized pyridazinone compounds and assayed them for inotropic action with special attention to their ability to lower central venous pressure in anesthetized dogs. We describe here the

RS-1893

Fig. 1. Chemical structure of RS-1893. 2-[2-Chloro-4-(2,3,4,5-tetrahydro-3-oxo-6-pyridazinyl)]-phenoxy-N-(2-morpholinoethyl)-acetamide.

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pharmacological and hemodynamic actions of one of these compounds, RS-1893, whose chemical structure is shown in Fig. 1.

Materials and Methods

Isolated dog ventricular muscle preparation: Hearts were excised from mongrel dogs of either sex (6 to 12 kg) anesthetized with sodium pentobarbital (30 mg/kg, i.v.). Trabeculae or thin papillary muscles were dissected from the free wall of the right ventricle. The ventricular muscle was mounted in a 20 ml organ bath containing bicarbonate-buffered Krebs-Henseleit solution (with 0.057 mM ascorbic acid and 0.027 mM disodium EDTA). The composition of the solution was as follows: 118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 24.9 mM NaHCO₃ and 11.1 mM glucose. The solution was bubbled with 95% O₂/5% CO₂ at 37°C. The muscle preparation was stimulated with a pair of platinum electrodes at a frequency of 0.5 Hz with square pulses of 5 msec duration and voltage about 20% above threshold. During an equilibration period of about 1 hr, the length of the muscle was adjusted to give the maximum contractile force. The force of isometric contraction was measured by means of a strain-gauge transducer (Nihon Kohden, TB-612T, AP-621G) and recorded on a thermal pen-writing oscillograph (NEC San-ei, Recti-Horiz-BK).

To ensure that the muscle functioned properly, it was exposed to 3x10⁻⁶ M of isoproterenol for 3 min. If the contractile force was not two-times the baseline value, the muscle was rejected. Isoproterenol was washed out of the bath and the muscle allowed about 60 min to stabilize. To establish cumulative concentration-response relationships, RS-1893 and other drugs were added to the baths at a 5 min interval.

Phosphodiesterase (PDE) preparation from dog hearts: The method of Thompson et al. (14) was used to isolate a crude cardiac preparation containing PDE activity. We dissected canine left ventricular and septal tissue and then minced and homogenized it using a Polytron. All procedures were performed at 4°C. The homogenate was centrifuged at 30,000×g for 60 min. The resulting supernatant was filtered and applied to a DEAE-cellulose column equilibrated with 70 mM sodium acetate/5 mM 2-mercaptoethanol (2ME) (pH 7.0). The column was then washed with 3 bed volumes of 70 mM sodium acetate/5 mM 2ME, after which the phosphodiesterases were eluted from the column using a continuous 70–1200 mM sodium acetate gradient (pH 7.0, containing 5 mM 2ME).

Assay of low Kₘ cAMP PDE (PDE-III) activity: Low Kₘ cyclic AMP PDE (PDE-III) was assayed by a modification of the method of Thompson and Appleman (15). The enzyme was incubated at 30°C in a total volume of 0.1 ml containing 40 mM Tris-HCl, pH 8.0, with 5 mM MgCl₂, 0.5 mM CaCl₂, 0.1 mg/ml bovine serum albumin and 0.25 μM [³¹⁴C]cAMP. Reactions were terminated after 10 min by immersion in boiling water for 60 sec. After cooling, 50 μl of snake venom (0.8 mg/ml) was added, and the mixture was incubated for 10 min at 30°C. Unreacted cAMP was removed by the addition of 1 ml of a 1:3 slurry of Amberlite IRP-58 (Rohm & Haas). Solutions were shaken and centrifuged at 1000×g for 5 min. Aliquots of the supernatant were counted.

Anesthetized dog preparation: Dogs of either sex weighing 8–16 kg were anesthetized with 30 mg/kg, i.v., of sodium pentobarbital, and 3–5 mg/kg/hr of intravenous infusion was continued. Following tracheal intubation, respiration was maintained with a positive pressure respirator. The left femoral artery and vein were cannulated for measuring blood pressure and injecting drugs, respectively. The left carotid artery was cannulated with a catheter-tip transducer (Millar, PC-350), and it was advanced into the left ventricle to measure left ventricular pressure (LVP), maximum LVdP/dt (LVdP/dt), and left ventricular end-diastolic pressure (LVEDP). A thermo-dilution balloon catheter (Gould H.B. Medical Products, SP5105) was inserted from the right femoral vein into the right ventricle and pulmonary artery. The right jugular vein was cannulated, and the cannula was advanced into the inferior vena cava to measure central venous pressure (CVP). Heart rate (HR) was measured using a tachometer triggered by an electrocardiogram. All variables were continuously recorded on a poly-
graph system (NEC San-ei, model 360). Cardiac output (CO) was intermittently measured with a cardiac output unit (Nihon Kohden, AH-611V, EQ-611V) after bolus injection of cold saline in a volume of 3.0 ml. Mean blood pressure (MBP) multiplied by CO gave cardiac work (CW) and MBP divided by CO gave total peripheral resistance (TPR).

Three studies were done. In one study, single intravenous doses of RS-1893, milrinone, CI-914, enoximone and amrinone were given to 4–6 dogs each to compare the cardiotonic actions of these agents. In another experiment, RS-1893 at the dose of 3, 7 and 20 μg/kg or milrinone at the dose of 10, 20 and 70 μg/kg was injected in a cumulative manner at a 10 min interval, and hemodynamic parameters such as HR, MBP, LVP, LVdP/dt, CO and CVP were measured. In a third study, propranolol hydrochloride was injected at a dose of 4 mg/kg, i.v., followed by continuous infusion of 0.13 mg/kg/min for 120 min. Thirty min after the initiation of propranolol infusion, 3, 7 and 20 μg/kg of RS-1893 was injected at a 10 min interval.

Unanesthetized instrumented dog preparation: Beagles of either sex weighing 9–12 kg were anesthetized with sodium pentobarbital 30 mg/kg, i.v. An endotrachial tube was inserted and ventilated with a constant-volume respirator (AIKA, R-60). The chest was opened through a ventrodorsal incision made in the 5th intercostal space under sterile conditions. To measure LVP and LVdP/dt, a catheter-tip pressure transducer (Nihon Kohden, TCP-2) was inserted from the apex of the left ventricle. The other end of the catheter was led under the skin and exteriorized through a small incision at the back. The chest was closed and negative intrapleural pressure was re-established. The left femoral artery and vein were cannulated with heparinized catheters (Toray Medical, Anthron) filled with saline, and the tubes were led under the skin to a small incision made on the back and exteriorized. After an intramuscular injection of 250,000 units of penicillin G, the dog was put on a jacket.

After recovery from surgery, 5 days were allowed for training the dogs to lie quietly. The dog was fasted 15 hr prior to the experiment. The pressure transducers were connected to transmitters (Nihon Kohden, ZB-671G) which were placed in the pockets of the dog jacket. The systemic blood pressure and LVP were monitored continuously with a telemetring system (Nihon Kohden, ZR-670G). Heart rate was measured by means of a cardiotachometer triggered by the LV pressure signal. The experimental dog could move and change position in the cage. The animal lay quietly most of the time. After a conditioning time of more than 2 hr, drug or placebo (lactose) was orally administered in a No. 3 gelatin capsule with a small amount of water. The gross behavior of the animal was observed during the whole experimental period.

Drugs: RS-1893, amrinone, milrinone and CI-914 were dissolved in 0.1 N HCl and diluted with physiological saline, except for oral administration. Enoximone was dissolved in 0.1 N NaOH and diluted with physiological saline. Drug solutions were freshly prepared each day.

Statistical analysis: All data points in the figures are values of the mean±S.E.M. Statistical significance was calculated using the paired Student's t-test. Values of P<0.05 were considered statistically significant.

Results

Isolated dog ventricular muscle preparation: RS-1893 increased the contraction of ventricular muscles in a concentration-dependent manner (Fig. 2). Positive inotropic effects reached a maximum within 5 min and remained so throughout the observation period. The concentration-response relation of RS-1893 showed a biphasic pattern: the inotropic response reached a plateau at 3×10⁻⁶–10⁻⁴ M, and the contractile force again increased at concentrations above 10⁻⁴ M. Doses required to increase the contraction by 10% of the maximum inotropic response to isoproterenol were calculated to be 0.24, 0.6, 4 and 20 μM for RS-1893, milrinone, CI-914 and amrinone, respectively.

Inhibition of PDE-III activity: As shown in Fig. 3, RS-1893 inhibited PDE-III, a low Kₘ cAMP specific form of the enzyme (IC₅₀= 0.12 μM). The agent was found to be approximately 15 times more potent than
milrinone (IC50=1.8 nM). Unlike milrinone, RS-1893 showed a biphasic inhibition of PDE-III.

Fig. 2. Effects of different cardiotonic agents on contractile force of dog trabeculae muscle preparations. Ordinates are the relative contractile force expressed as percent of the maximum response to isoproterenol (3×10⁻⁶ M). Values represent the mean±S.E.M. of 4 to 8 experiments.

Fig. 3. Effects of RS-1893 and milrinone on low Kₐₐₐ cAMP phosphodiesterase (PDE-III) activity. Each point represents the means of duplicate experiments.

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Intravenous administration in anesthetized dogs: Figure 4 illustrates the changes in HR, LVdP/dt and MBP produced by 3 μg/kg of RS-1893 in anesthetized dogs. LVdP/dt increased by 24% and HR increased slightly but significantly within 1 min, although MBP did not change significantly. The inotropic effect of RS-1893 reached a maximum within 1 to 2 min after administration and lasted for 30 min. Figure 5 shows dose-response relations for changes in LVdP/dt following a single intravenous administration of various cardiotonics in anesthetized dogs. There were clear dose-response relations for all the compounds. The in vivo cardiotonic action of RS-1893 was about 3, 10, 80 and 200 times more potent than those of milrinone, CI-914, enoximone and amrinone, respectively.

In another series of anesthetized dogs, RS-1893 was administered cumulatively at a 10 min interval. LVdP/dt increased in a dose-related manner, and the increase lasted for more than 60 min at the maximum dose used: 30 μg/kg, i.v. (Fig. 6, upper panel). HR increased, but to a lesser extent than LVdP/dt. The duration of the positive chronotropic action was also less than that of the inotropic action. MBP did not change significantly up to 10 μg/kg, i.v., of RS-1893, but was lowered significantly at a total dose of 30 μg/kg, i.v. The reduction of MBP lasted for 45 min at 30 μg/kg, i.v. CVP and TPR decreased significantly at 3 μg/kg of RS-1893, and these actions were dose-dependent and long-lasting. CO measured by the thermo-dilution method increased even at the lowest dose: 3 μg/kg, i.v. (Fig. 6, lower panel).

Similar experiments were done with milrinone as well. The maximum changes in LVdP/dt, MBP, HR, CVP, CO, CW and TPR produced by RS-1893 and milrinone were plotted against the dose in Fig. 7. The figure shows that RS-1893 was 3 times more potent than milrinone in increasing LVdP/dt and in decreasing MBP and TPR. On the other hand, RS-1893 was 10 times more
potent than milrinone in lowering CVP and increasing HR.

**Oral administration in conscious dogs:** RS-1893, milrinone or placebo was orally administered in conscious dogs chronically instrumented with a catheter-tip pressure transducer implanted into the left ventricle. Control dogs (N=6) administered with lactose did not show any significant change in all parameters during the 8 hr observation period (Fig. 8, upper panel). The middle panel of Fig. 8 illustrates percent changes in $LV\dot{d}P/dt$, HR and MBP produced by RS-1893. At a dose of 30 $\mu$g/kg, $LV\dot{d}P/dt$ increased significantly 12 min after administration, reaching a maximum in 1–3 hr (20%). The increase of $LV\dot{d}P/dt$ lasted about 8 hr. HR increased by about 12% and returned to near control values at 3–4 hr. MBP decreased (-6%) only temporarily.

At 100 $\mu$g/kg, $LV\dot{d}P/dt$ increased more markedly (Fig. 8, lower panel). The increase of
LVdP/dt reached a maximum in 2 hr (37%) and lasted more than 8 hr. HR increased by about 17%, but the increase of HR was smaller than that of LVdP/dt and lasted for a shorter time (3–4 hr). MBP showed a significant and sustained decrease. No abnormality was observed in their gross behavior.

Figure 9 shows the effects of an oral administration of milrinone. Milrinone at 0.3 and 1.0 mg/kg produced increases in LVdP/dt that were similar to those produced by RS-1893 at 0.03 and 0.1 mg/kg, respectively, but the cardiotonic action of milrinone lasted for a shorter time than that of RS-1893.

Propranolol-induced heart failure: Intravenous administration of propranolol in a single large dose (4 mg/kg) followed by a continuous infusion (0.13 mg/kg/min) caused marked and sustained decreases in LVdP/dt (−52%) and CO (−23%), while CVP and LVEDP increased significantly (+49% and +730%). MBP and HR were reduced by propranolol (Fig. 10).

After 30 min, when propranolol heart failure was established, RS-1893 was intra-
venously administered at doses of 3, 7 and 20 µg/kg at a 10 min interval. RS-1893 caused immediate and dose-dependent increases in LvdP/dt and CO and caused significant reductions in LVEDP, CVP and TPR. MBP decreased and HR increased in a dose-related manner.

The control group which was treated with propranolol but not administered RS-1893 showed a heart failure similar to the other group. As shown by the open circles, all parameters remained stable for a 100 min observation period, except for MBP which slightly decreased with time.

Figure 11 shows LVEDP-CO relations in the normal heart and the failing heart before and after intravenous administrations of RS-1893 at different doses. Propranolol treatment caused a right and downward shift of the relation, indicating a severe cardiac failure. This shift was reversed by RS-1893 in a dose-related manner.

**Discussion**

RS-1893 caused a concentration-depend-ent increase in contraction of isolated ventricular muscles. Comparison of the doses that produced a 10% increase of myocardial contraction, revealed that RS-1893 was more potent than the reference compounds used in this study: 3, 16 and 80 times more potent than milrinone, CI-914 and amrinone, respectively. Unlike the other compounds, however, RS-1893 showed a biphasic positive inotropic action (Fig. 2). The first inotropic phase started from 10^{-8} M and reached a plateau at 3\times10^{-6} M, which was about 30% of the maximum response to isoproterenol (3\times10^{-6} M). The second phase started from 10^{-4} M, and the maximum inotropic action achieved with RS-1893 was comparable to those obtained with the other compounds.

The minimum concentration of the second phase of inotropic action in vitro was 10^{-4} M (=30 µg/ml), which was never achieved in vivo unless such an extremely high dose as 3 mg/kg, i.v., was administered. Thus, the in vivo inotropic action observed at doses up to 0.03 mg/kg, i.v., was solely due to the first phase inotropic action in vitro. Indeed, the order of potency in vitro, when compared by the doses that produce a 10% increase of cardiac contraction, paralleled that in vivo: RS-1893 > milrinone > CI-914 > amrinone.

RS-1893 inhibited PDE-III isolated from dog ventricle (Fig. 3). The inhibitory action of the agent, compared by the doses required to inhibit PDE-III by 50%, was about 15 times more potent than that of milrinone. Whereas a monophasic pattern of inhibition was achieved by other compounds, such as milrinone and amrinone (16, 17), only RS-1893 showed a biphasic pattern of inhibition. The first inhibition phase started from about 10^{-8} M and reached a plateau at 2.5\times10^{-7} M, which was about 65% inhibition. The second phase started from 2.5\times10^{-5} M, and a full inhibition of PDE-III activity was achieved at 2.5\times10^{-3} M. This profile in enzyme inhibition resembled the in vitro pharmacological profile: unlike other compounds, RS-1893 formed a plateau in its in vitro inotropic action. These data suggest that inhibition of PDE-III underlies the cardiotonic activity of RS-1893, as has been suggested for many other cardiotonics (18–20). However, other mechanisms can not be excluded as structurally related
compounds such as pimobendan and sulmazole have been reported to cause supersensitivity to Ca\(^{2+}\) (4, 21, 22).

The biphasic inotropic action or partial inhibition of PDE have been observed with other inotropic compounds. Alousi et al. (23) reported that milrinone showed a biphasic inotropic action in isolated guinea pig papillary muscle, which was not confirmed in the present study using isolated dog heart preparation. Pimobendan has been shown to produce a partial inhibition of PDE (16). It is noteworthy that RS-1893 and pimobendan share a common chemical structure.

An oral administration of RS-1893 at a dose of 30 µg/kg produced a significant increase of LVdP/dt 12 min after administration. The increase of LVdP/dt reached a maximum (20%) in 1–3 hr, which was almost comparable in magnitude with the maximum response obtained after intravenous administration at the dose of 3 µg/kg. These data suggest that RS-1893 was readily absorbed from the gastro-intestinal tracts. This consideration is supported by the fact that RS-1893 was 3 times more potent than milrinone in intra-

Fig. 8. Effects of orally administered placebo (lactose) and RS-1893 in a No. 3 gelatin capsule on LVdP/dt, heart rate (HR) and mean arterial blood pressure (MBP) in chronically instrumented conscious dogs. Upper panel represents the placebo treated group (N=6). Middle and lower panels represent either the 30 or 100 µg/kg of RS-1893 treated groups (N=5). Asterisks indicate P<0.05, compared to the pre-treated value.
Fig. 9. Effects of orally administered milrinone on LVDp/dt, heart rate (HR) and mean arterial blood pressure (MBP). Chronically instrumented dogs received either 0.3 or 1.0 mg/kg of milrinone in a No. 3 gelatin capsule. Asterisks indicate \( P < 0.05 \), compared to the pre-treated value (N=4).

Fig. 10. Effects of RS-1893 on propranolol induced heart failure in anesthetized dogs. Closed circles represent the RS-1893 group and open circles the control group. Values are means±S.E.M. (N=5). Asterisks indicate \( P < 0.05 \) by the paired t-test. Pretreatment values of the RS-1893 group for LVDp/dt, CO, LVEDP, HR, MBP, CVP and TPR were 2490±350 mmHg/sec, 1.81±0.16 l/min, 0.3±0.7 mmHg, 127±11 beats/min, 138±5 mmHg, 5.2±1.2 cmH2O and 77±5 mmHg•min/l, respectively.
The reduction of CVP was observed at the lowest dose of RS-1893 used and persisted for more than 1 hr after injection of the highest dose: 30 µg/kg, i.v. The most likely explanation for the reduction of CVP is venous vasodilation. In fact, RS-1893 increases hindlimb volume in anesthetized dogs, suggesting that the agent dilates venous blood vessels. The positive inotropic action may partly account for the reduction of CVP. However, this is a less likely mechanism than venous vasodilation, because the increase in LVdP/dt did not parallel the reduction of CVP: the once increased LVdP/dt declined rapidly with time, while CVP stayed lowered for the 60 min observation period. Milrinone also lowered CVP, but to a lesser extent than RS-1893 did.

Venous dilators such as nitroglycerin and ISDN are used for the treatment of congestive heart failure (10, 11). They decrease preload and reduce ventricular size, thus improving cardiac efficiency. At the same time they reduce cardiac output and may decrease tissue perfusion. Though RS-1893 has venous vasodilator action, there seems to be no danger of diminishing tissue blood flows as evidenced by the increase in cardiac output. Thus, combination of inotropic action and venous and arterial vasodilator actions in one molecule may produce a beneficial hemodynamic change in the treatment of congestive heart failure.

Finally, we evaluated the cardiotonic action of RS-1893 in propranolol-induced heart failure. In this model of heart failure, the increases of LVdP/dt and HR and decreases of CVP, MBP and TPR were of almost the same magnitude as in the normal heart, indicating that beta-stimulant action is not involved in the cardiotonic action of RS-1893. However, the increase of CO produced by RS-1893 was greater in the failing heart than in the normal heart. The cardiac function curve, i.e., relationship between LVEDP and CO, in the failing heart was normalized by the agent at 10 µg/kg, i.v.

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