Improvement of Pentobarbital-Induced Heart Failure by MCI-154, a Novel and Potent Cardiotonic Agent, in the Dog Heart-Lung Preparation

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Abstract—The efficacy of MCI-154, a new pyridazinone cardiotonic agent, in improving heart failure was assessed in dog heart-lung preparations in which cardiac function had been severely depressed by pentobarbital. MCI-154 in doses of 10–100 μg improved the cardiac function curve and restored it to the control level at 100 μg. At this dose, MCI-154 neither produced an increase in heart rate beyond the control value nor induced arrhythmias. The effects of MCI-154 were not affected by atenolol, a cardioselective β₁-blocker. These results indicate that MCI-154 would be of potential use in the treatment of heart failure.

MCI-154, 6-[4-(4'-pyridyl)aminophenyl]-4,5-dihydro-3(2H)-pyridazinone hydrochloride (Fig. 1), is a newly developed positive inotropic drug which is structurally different from cardiac glycosides and α₂-stimulants. This drug exerts a pronounced positive inotropic effect in anesthetized and conscious dogs and in isolated cardiac muscles of various mammalian species, while producing only a moderate increase in atrial or heart rate (1–3). These effects in vivo were not antagonized by propranolol, ruling out any possible involvement of β-adrenergic mechanisms in its positive inotropy (1–3). The drug does not inhibit Na⁺,K⁺-ATPase (1, 2, 4). Unlike most new nonglycoside and nonsympathomimetic cardiotonic agents, the drug scarcely increases intracellular cyclic AMP in dog ventricular muscle, ruling out the major role of an increase in intracellular cyclic AMP in its positive inotropy (4). MCI-154 has been found definitely to increase the sensitivity to calcium ions of contractile proteins in chemically skinned papillary muscles of the guinea-pig ventricle (5, 6). Thus, it is a drug with a novel mechanism of action.

The drug has been shown to ameliorate
heart failure produced by excessive doses of propranolol or verapamil in the in situ heart of anesthetized dogs (2, 3). However, in these heart failure models, it is difficult to evaluate the drug effect on the cardiac function curve. The present experiments were designed to obtain more detailed information about such an effect by the use of dog heart-lung preparations as an experimental model of heart failure.

**Materials and Methods**

Thirteen heart-lung preparations were made from mongrel dogs of either sex, weighing 9–12 kg, anesthetized with sodium pentobarbital (30 mg/kg, i.v.). The preparations were essentially the same as those described previously (7, 8). The extracorporeal blood circulation circuit consisted of silicone rubber tubing, a Windkessel chamber, a Starling pneumatic resistor (giving a pressure load comparable to the mean systemic arterial blood pressure), a heat exchanger (to control the temperature of the circulating blood) and a blood reservoir. The blood reservoir had been primed with 500–550 ml of arterial blood obtained from other mongrel dogs anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and given calcium heparin (500 units/kg, i.v.). The level of blood in the reservoir was maintained at 5 cm above the level of the tricuspid valve (basal conditions). Aortic blood pressure (given by the Starling pneumatic resistor) was maintained at 80 mmHg and monitored with a pressure transducer (Nihon Kohden, MPU-0.5) through a side arm of the aortic cannula. Cardiac output (which did not include coronary blood flow) was measured with the flow probe of an electromagnetic flowmeter (Nihon Kohden, MFV-2100) interposed between the aortic cannula and the Starling pneumatic resistor. Left and right atrial pressures were measured with pressure transducers (Gould Statham, P23ID). Left ventricular pressure (LVP) was measured with a Mikro-tip® catheter pressure transducer (Millar Instruments) inserted into the left ventricle via the left subclavian artery. The maximum rate of rise of LVP (LV dP/dt max) was obtained with an electronic differentiator (San-ei Instrument, 1323). Heart rate was measured with a cardiotachometer (San-ei Instrument, 1321). Atrioventricular (AV) conduction time was measured with an AV interval counter (Data Graph, HT-31) at a resolution of 1 msec. To determine cardiac function curves which relate cardiac output to left atrial pressure, the level of blood in the reservoir was elevated stepwise by 5 cm for 30 sec up to 10 cm above the basal level. Such a procedure is designated as a competence test. To produce heart failure, sodium pentobarbital was administered into the blood reservoir initially at 50 mg and then by 25 mg steps, until cardiac output was decreased to about 60% of the control level.

MCI-154, 6-[4-(4'-pyridyl)aminophenyl]-4,5-dihydro-3(2H)-pyridazinone hydrochloride (Mitsubishi Chemical Industries), was dissolved in 0.9% NaCl at a concentration of 10 mg/ml as a stock solution. The stock solution was diluted with 0.9% NaCl immediately before use and administered into the blood reservoir. (-)-Isoproterenol hydrochloride (Sigma) and atenolol (ICI) were dissolved in 0.9% NaCl. All doses referred to cumulative ones as bases.

Values are given in terms of the mean±S.E., unless otherwise stated. Differences between mean values were analyzed by Student's t-test and taken to be significant when P values were less than 0.05.

**Results**

Effects of MCI-154: Seven heart-lung preparations were used. The basal values of cardiac variables are presented in Table 1A. Before the administration of pentobarbital, left atrial pressure and cardiac output were 6.8±0.9 mmHg and 499±27 ml/min, respectively, under basal conditions. On elevation of the level of blood in the reservoir to 10 cm above the basal level, left atrial pressure and cardiac output were 9.7±1.3 mmHg and 881±71 ml/min, respectively. Consequently the cardiac function curve was shifted down-
Table 1. Cardiac variables of the dog heart-lung preparation before (control) and after administration of pentobarbital (A) and before (control) and after administration of isoproterenol, atenolol and pentobarbital (B)

|                  | Cardiac output (ml/min) | Left atrial pressure (mmHg) | Right atrial pressure (cmH₂O) | LV dP/dt max (mmHg/sec) | Heart rate (beats/min) | AV conduction time (msec) |
|------------------|-------------------------|------------------------------|-------------------------------|-------------------------|------------------------|--------------------------|
| **A (n=7)**      |                         |                              |                               |                         |                        |                          |
| Control          | 499±27                  | 6.8±0.9                      | 2.8±0.4                       | 2157±129                | 165±6                  | 107±7                    |
| Pentobarbital    | 306±32*                 | 9.7±1.3*                     | 4.1±0.2*                      | 1403±80*                | 136±6*                 | 119±8                    |
| 121±80 (S.D.) mg |                         |                              |                               |                         |                        |                          |
| **B (n=6)**      |                         |                              |                               |                         |                        |                          |
| Control          | 648±86                  | 6.4±0.6                      | 2.6±0.3                       | 1672±125                | 159±8                  | 99±6                     |
| Isoproterenol (1 µg) | 743±90*               | 5.0±0.5*                     | 2.1±0.2*                      | 2182±208*               | 194±15*                | 90±6*                    |
| Atenolol (1 mg)  | 623±88                  | 6.6±0.5                      | 2.7±0.3                       | 1638±128                | 159±10                 | 102±6                    |
| Pentobarbital    | 368±90*                 | 10.9±1.4*                    | 4.4±0.4*                      | 1132±121*               | 148±7*                 | 110±5*                   |
| 88±44 (S.D.) mg  |                         |                              |                               |                         |                        |                          |

*P<0.05, against the respective control values.
wards and to the right by pentobarbital. Following the addition of MCI-154 (10–100 μg), the cardiac function curve depressed by pentobarbital was shifted upwards and to the left in a dose-dependent manner (Figs. 2 and 3). At 100 μg MCI-154, almost complete improvement of the depressed cardiac function curve was accomplished, judging from the cardiac function curve.

Under basal conditions, right atrial pressure rose from 2.8±0.4 cmH2O in the control to 5.3±0.6 cmH2O in heart failure. The elevated right atrial pressure was lowered with MCI-154 (10–100 μg) in a dose-dependent manner.

The decrease in LV dP/dt max in the failing heart and its improvement with MCI-154 are presented in Figs. 2 and 4. LV dP/dt max was restored to its control value with a dose of 30–100 μg MCI-154. (Figs. 2 and 4).

Changes in heart rate and AV conduction time are shown in Figs. 2 and 4, respectively.

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Fig. 2. Effects of MCI-154 on cardiac output, left atrial pressure, right atrial pressure, LV dP/dt, heart rate and AV conduction time.

Fig. 3. Cardiac function curves determined with MCI-154 in dog heart-lung preparations. Data points are means±S.E. of 7 preparations. Control (○), heart failure (●). MCI-154 in cumulative doses: 10 (▵), 30 (▲), 100 μg (□).

Heart rate was 165±6 beats/min in the control and decreased to 136±6 beats/min in
the failing heart. Heart rate was slightly increased with increasing doses of MCI-154; however, with 100 μg MCI-154, heart rate was still lower than the control value (before pentobarbital) (Figs. 2 and 4). Even with 300 μg, heart rate failed to recover to the control value (data are not shown). AV conduction time was prolonged from 107±7 msec in the control to 119±8 msec in the failing heart, which in turn was shortened by MCI-154. No arrhythmias were induced by MCI-154 at any of the doses used.

Effects of MCI-154 in the presence of atenolol: Six other heart-lung preparations were used. The basal values of cardiac variables are shown in Table 1B. The cardiac function curve was shifted upwards and to the left with 1 μg of isoproterenol, and LV dP/dt max was increased from 1672±125 mmHg/sec to 2182±208 mmHg/sec (by 30% of control), and heart rate was also increased from 159±8 beats/min to 194±15 beats/min (by 22% of control). Under these conditions, 1 mg of atenolol was administered to the blood reservoir. With this dose of atenolol, cardiac variables resumed values not significantly

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**Fig. 4.** Effects of MCI-154 on LV dP/dt max (A), right atrial pressure (B), heart rate (C) and AV conduction time (D). Data points are means±S.E. of 7 preparations. C, control; F, failure produced by pentobarbital. *P<0.05.

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**Fig. 5.** Cardiac function curves determined with isoproterenol and atenolol and MCI-154. Data points are means±S.E. of 6 preparations. Control (○—○), heart failure (●—●), MCI-154 in cumulative doses: 10 (△—△), 30 (▲—▲), 100 μg (■—■). Isoproterenol (1 μg) (□——□). Atenolol (1 mg) (▲—▲).
different from the controls (Table 1B). After the administration of atenolol, pentobarbital (88±44 mg, mean±S.D.) caused depression of cardiac function (Fig. 5). Following the administration of MCI-154 (10–100 μg), the cardiac function curve depressed by pentobarbital was shifted upwards and to the left in a dose-dependent manner so that at 100 μg, it was restored to its control (Fig. 5). At 100 μg of MCI-154, LV dP/dt max, right atrial pressure and heart rate were restored to the respective control values, and AV conduction time was rather shortened beyond the control value (data not shown).

Discussion

In the present experiments, MCI-154 (10–100 μg) clearly improved the cardiac function which had been severely depressed by pentobarbital. At 100 μg of MCI-154, cardiac function was almost restored to the control state, but heart rate remained a little lower than the control. At this dose of MCI-154, AV conduction was slightly accelerated. Nevertheless, in all the doses examined, no arrhythmias were induced. Thus, there is a definite force-rate separation in MCI-154 so far as its effects were assessed in the pentobarbital-induced heart failure model of the dog heart-lung preparation.

MCI-154 (2, 3) has been shown to be more potent than amrinone, a prototype new cardiotonic drug (9, 10). When compared in the doses that restored the depressed cardiac function curve to the control in the dog heart-lung preparation, the cardiotonic potency of MCI-154 appears to be more than 300 times that of amrinone, since the latter failed to restore the depressed cardiac function curve to the control even at the dose of 10 mg (11). Even with higher doses of amrinone, it still failed to restore the depressed cardiac function curve to the control, since 3 and 10 mg were nearly equi-effective (11). In this respect, MCI-154 appears to be superior to amrinone as a cardiotonic drug. The improving effect of MCI-154 in the pentobarbital-induced failing heart was not affected by the β₁-selective blocker atenolol. This indicates that the effect of MCI-154 is not mediated via β-receptors, as has been shown by previous studies (1–3).

In short, the present study demonstrated that experimental heart failure was improved completely by MCI-154 without increase in heart rate. No arrhythmias were induced. In addition, MCI-154 has been shown to have a potent vasodilator action (1–3). These taken together suggest that it would be worthwhile to subject MCI-154 to clinical trials in the treatment of patients with heart failure.

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