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Positive inotropic effect of MCI-154 on hypertrophied heart of rats and its mechanisms

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Abstract: **AIM** To explore if there is a differential effect of MCI-154 〔6-[4-(4′-pyridylamino) phenyl]-4,5-dihydro-3(2*H*)-pyridazinone hydrochloride trihydrate〕, a calcium sensitizer, on hypertrophied hearts compared with normal hearts and the possible mechanisms underlying it. **METHODS** Functions of rat hypertrophied heart were investigated using Langendorff perfusion. Ca^{2+} transient and cell shortening in isolated cardiac myocytes were simultaneously measured using ion imaging system. **RESULTS** ① MCI-154 100 – 400 $\mu mol \cdot L^{-1}$ improved cardiac performance assessed by active systolic pressure (left ventricular systolic pressure minus left ventricular diastolic pressure) and $+dp/dt_{max}$ in hypertrophied hearts as well as in normal ones, and $-dp/dt_{max}$ tended to increase slightly with no statistical significance; ② MCI-154 concentration-dependently increased cell shortening in hypertrophied myocytes from $(4.8 \pm 1.7)\mu m$ of control to (5.6 ± 1.5), (7.6 ± 1.7) and $(10.9 \pm 1.7)\mu m$ at 1, 10 and 100 $\mu mol \cdot L^{-1}$, respectively, with unaltered Ca^{2+} transient amplitude and Ca^{2+} transient decline assessed by time at 50% and 90% restoring. **CONCLUSION** MCI-154 exerted positive inotropic effect concentration dependently *via* calcium sensitizing on hypertrophied rat heart as on normal rat heart.

Key words: calcium sensitizer; MCI-154; heart hypertrophy; myocardial contraction; calcium, cytosolic

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MCI-154, 6-[4-(4′-pyridylamino) phenyl]-4,5-dihydro-3(2*H*)-pyridazinone hydrochloride trihydrate, is a novel positive inotropic agent that increases cardiac contractility of guinea-pigs, rabbits, canines and rats[1-4]. It prominently causes the leftward shift of the Ca^{2+} concentration-tension relationship in skinned cardiac fibers[5], which suggest that it increase the Ca^{2+} sensitivity of the contractile apparatus. However, some investigators reported that calcium sensitizers produced different effects in pathologic myocardium compared with normal myocardium[6,7]. It is, therefore, important to investigate the effects of MCI-154 on pathologic hearts. Because left ventricular hypertrophy is a very common adaptive response accompanied by decreased compliance and diastolic function, the cardiac hypertrophied models were adopted to observe the effect of MCI-154 on hypertrophied myocardium. In this study, we investigated the effects of MCI-154 on heart function of hypertrophied rat hearts using Langendorff perfusion apparatus. In order to explore the mechanisms underlying it, Ca^{2+} transient and cell shortening in hypertrophied ventricular myocytes of rats were simultaneously measured using ion imaging technique.

1 MATERIALS AND METHODS

1.1 Preparation of animal models

Myocardial hypertrophy in rats was induced by abdominal aortic banding[8]. Male Wistar rats weighing 160 – 180 g provided by Experimental Animal Center of Shanxi Medical University were anesthetized with 1% sodium pentobarbital (3 $mL \cdot kg^{-1}$, ip) and the abdomen opened by midline incision. At 1 cm segment of aorta was dissected free and 0.8 mm diameter needle placed on its

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anterior surface. Surgical silk was then tightly banded around the needle and aorta to provide a uniform degree of constriction. The wire was withdrawn from the ligature so that the diameter of the remaining orifice of the ligature approximate that of the wire. Then the abdomen was closed and the animals were allowed for intake of food and water *ad libitum*. Blood pressure was monitored weekly after the operation. Only animals with systolic blood pressure > 20 kPa four weeks after operation were used for studies.

1.2 Measurement of cardiac function

Male Wistar rats were sacrificed by decapitation and hearts were rapidly excised and placed in ice-cold Tyrode's solution. Hearts were mounted on a Langendorff perfusion apparatus where they were perfused with Tyrode's solution equilibrated with 100% O_2 at 36℃ (pH 7.4) at a flow rate of 8 – 10 mL·min^{-1} according to the size of hearts with a HL-2 peristaltic pump (Shanghai Scientific Experiment Factory). The heart was paced at a rate of 200 min^{-1}, 200% of threshold voltage with a stimulator (NHON KOHDEN) *via* a silver wire placed into the right ventricle and secured to the pulmonary artery outflow. A latex balloon attached to plastic catheter was installed into the left ventricle through the mitral valve and filled with perfusate solution and connected to a pressure transducer (XHYP-110). Hemodynamic changes were recorded on a polygraph recording system (MS 302, Guangzhou Pharmacological Academy). Throughout the experiment, the balloon volume was not altered so that the heart functioned isovolumically. The perfusate contained (in mmol·L^{-1}): NaCl 135, KCl 5.4 , $MgCl_2$ 1.0, glucose 10, HEPES 5, NaH_2PO_4 0.33, $CaCl_2$ 1.8, pH 7.4 adjusted with NaOH. After a 30 min stabilization period, hearts were perfused with MCI-154 at concentrations from 100 to 400 μmol·L^{-1}.

1.3 Isolation of cardiac myocytes

It was performed as described by Lu, *et al*[9] and Cui, *et al*[10]. The composition of the Ca^{2+}-free Tyrode's solution was (in mmol·L^{-1}): NaCl 104, KCl 10.0 , $MgSO_4$ 1.0, NaH_2PO_4 1.0, HEPES 5 and glucose 20.0 (pH 7.4 adjusted with NaOH). Myocytes were kept in fresh Krebs buffer (KB) solution at room temperature (22℃) for 1 h before use. The KB solution was composed of (in mmol·L^{-1}): KOH 85, *L*-glutamic acid 50, KCl 30, taurine 2.0, KH_2PO_4 30, $MgSO_4$ 1.0, HEPES 10, glucose 10, and EGTA 0.5, pH was adjusted to 7.4 with KOH.

1.4 Measurements of cell contraction and Ca^{2+} transient

Measurements of cell Ca^{2+} transient was performed using ion imaging system with charge coupled digital camera (Tillphotonics, Germany) as described by Cui, *et al*[10]. Briefly, after loading with Fura-2, myocytes were placed in an open-flow chamber mounted on a Zeiss inverted epifluorescence microscope. The chamber was equipped with Pt electrodes to allow electrical field stimulation of the myocytes within the field of view. A stimulation frequency of 0.5 Hz was used throughout all experiments. Cells were perfused continuously at a flow rate of 2 mL·min^{-1} with modified Tyrode's solution containing 0.2% bovine serum albumin and 1.0 mmol·L^{-1} $CaCl_2$. A BPS-4 switching system (Ala Scientific Instruments Inc., American) was used to interchange among four buffer reservoirs for addition of drugs. Cells were selected for study based on their overall physical appearance (elongated rods with well-defined sarcomere structure and no blebs), quiescence in the absence of stimuli and their ability to contract in response to electrical field stimulation. Ca^{2+} transient is expressed as F340 nm/F380 nm (F340/F380). Cell contraction was expressed by cell shortening(μm), *i.e.* the quiescent length of the cell minus the shortest length of the cell during its contraction[11]. Ca^{2+} sensitivity was the ratio of calcium transient and cell shortening of the same cell[12].

1.5 Drugs and chemicals

MCI-154 (purity > 99.5%) was synthesized by the Pharmaceutical College, the Second Military Medical University, Shanghai. Fura-2/AM and HEPES were purchased from Sigma Chemical Co. Fura-2/AM was dissolved in dimethylsulphoxide and diluted in perfusates to 5 μmol·L^{-1}. Other

Tab 1. Morphological and other parameters in rats with heart hyprtrophy

Group	BW/g	SBP/kPa	WTLV/mm	CALV/mm^2	CA/mm^2
Normal	280 ± 15	15.8 ± 1.3	1.9 ± 0.1	13.5 ± 2.0	0.010 ± 0.001
Hypertrophy	283 ± 18	24.6 ± 2.1*	3.1 ± 0.4*	8.6 ± 1.6*	0.020 ± 0.002**

Rats with heart hypertrophy were induced by banding the abdominal aorta at suprarenal. Tests were done four weeks after operation. BW: body weight; SBP: systolic blood pressure; WTLV: wall thickness of left ventricle; CALV: cavity area of left ventricle; CA: cell area. $\bar{x} \pm s$, $n = 6$. * $P < 0.05$, ** $P < 0.01$, compared with normal group.

reagents are AR grades.

1.6 Statistical analysis

Data were expressed as $\bar{x} \pm s$. Statistical significance was determined by t test.

2 RESULTS

2.1 Hypertrophied hearts

The morphological changes of hypertrophied hearts were the markedly thickened wall with decreased size of ventricular cavity (Tab 1). There was significant difference in wall thickness and cavity area of left ventricular between hypertrophied rats and normal ones.

2.2 Effects of MCI-154 on cardiac function in hypertrophied rat hearts

The result showed that MCI-154 improved cardiac systolic function in a concentration-dependent manner without worsening cardiac relaxation (Tab 2). MCI-154 at 400 μmol·L^{-1} significantly increased left ventricular active systolic pressure (left ventricular systolic pressure minus left ventricular diastolic pressure) and $+dp/dt_{max}$, while $-dp/dt_{max}$ tended to be slightly increased with no statistical significant.

Tab 2. Effects of MCI-154 on cardiac function in hypertrophied heart of rats

MCI-154 /μmol·L^{-1}	LVSP − LVDP /kPa	$+(dp/dt_{max})$ /kPa·s^{-1}	$-(dp/dt_{max})$ /kPa·s^{-1}
0	10.6 ± 2.0	205 ± 16	113 ± 14
100	12.1 ± 2.2	215 ± 14	122 ± 17
200	12.6 ± 2.0	224 ± 17	128 ± 12
400	13.7 ± 2.0*	230 ± 18*	130 ± 12

MCI-154 was progressively added in Tyrode's solution to different final concentration in 15 min intervals. LVSP: left ventricular systolic pressure; LVDP: left ventricular diastolic pressure; LVSP − LVDP: active systolic pressure. $\pm dp/dt_{max}$: maximal rate of rise and decline of ventricular pressure. $\bar{x} \pm s$, $n = 6$. * $P < 0.05$, compared with 0 μmol·L^{-1}.

2.3 Effects of MCI-154 on cell contraction and Ca^{2+} transient in hypertrophied ventricular myocytes of rats

Simultaneous recording of cell contraction and Ca^{2+} transient was performed using ion imaging system. The result showed that MCI-154 concentration-dependently increased cell shortening with unaltered Ca^{2+} transient (Tab 3). Ca^{2+} sensitivity derived from the ratio of calcium transient and cell shortening increased concentration-dependently. Fig 1 showed a representative example of the effects of MCI-154 on Ca^{2+} transient (upper line) and cell length (lower line) in hypertrophied myocytes.

Tab 3. Effects of MCI-154 on Ca^{2+} transient and cell contraction in hypertrophied myocytes

MCI-154 /μmol·L^{-1}	Peak value of Ca^{2+} transient (F340/380)	Shortening /μm	Ca^{2+} sensitivity /μm^{-1}
0	178 ± 29	4.8 ± 1.7	39.3 ± 8.8
1	192 ± 29	5.6 ± 1.5	35.3 ± 5.2
10	175 ± 21	7.6 ± 1.7*	23.7 ± 3.6**
100	180 ± 38	10.9 ± 1.7**	16.6 ± 2.9**

MCI-154 was added in Tyrode's solution to different final concentration. Ca^{2+} sensitivity was the ratio of calcium transient and cell shortening of the same cell. $\bar{x} \pm s$, $n = 6$. * $P < 0.05$, ** $P < 0.01$, compared with control.

Tab 4 showed that MCI-154 had no effect on Ca^{2+} transient decline kinetics assessed by TR_{50} and TR_{90}.

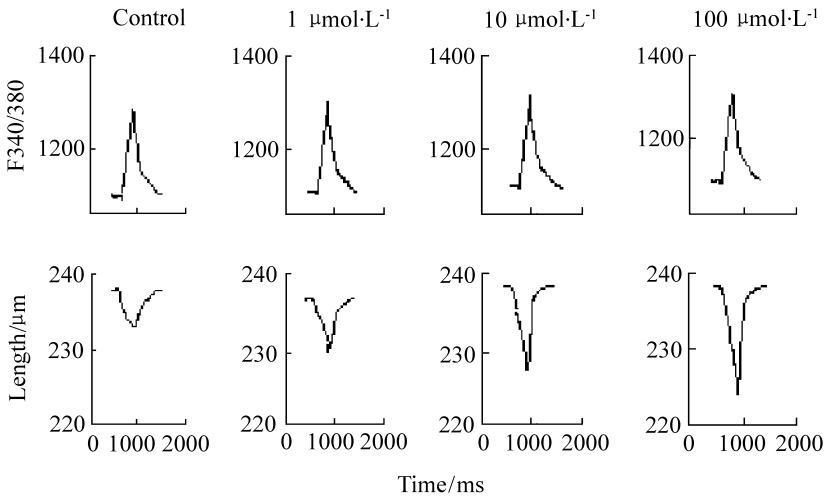

Fig 1. The representative traces of effects of MCI-154 on the simultaneous recordings of Fura-2 fluorescence ratio (upper traces) and cell shortening (lower traces) in hypertrophied rat ventricular myocytes.

Tab 4. Effects of MCI-154 on Ca^{2+} transient duration in hypertrophied myocytes

MCI-154/μmol·L^{-1}	TP/ms	TR_{50}/ms	TR_{90}/ms
0	105 ± 8	208 ± 24	411 ± 8
1	105 ± 8	205 ± 24	409 ± 9
10	104 ± 8	201 ± 24	406 ± 8
100	100 ± 10	192 ± 24	401 ± 8

MCI-154 was added in Tyrode's solution to different ultimate concentration. TP: time to peak of Ca^{2+} transient; TR_{50}: time to 50% restoring of Ca^{2+} transient; TR_{90}: time to 90% restoring of Ca^{2+} transient. $\bar{x} \pm s$, $n = 6$ cells from 6 rats. $P > 0.05$, compared with control.

3 DISCUSSION

The two general mechanisms of cardiotonic agents are increasing intracellular calcium concentration or alter the responsiveness of myofilament to Ca^{2+}. The major currently used cardiotonic agents, including the cardiac glycosides, catecholamine and phosphodiesterase (PDE) inhibitors, act through the former mechanism. More recently, agents which increase the strength of contraction by increasing the response of contractile proteins to Ca^{2+} [2,13-15] have been developed. MCI-154 is such a calcium sensitizer. Previous studies have shown that it exerts positive inotropy in isolated ventricular muscles from various mammalian species[2,16] and in canine pathological models of pacing-induced heart failure[17], ischemia[18,19], acidosis and stunned myocardium[20]. In this study, we showed that MCI-154 caused improvement of cardiac performance in hypertrophied hearts.

Up to now, there was no study about the mechanisms of effects of MCI-154 on hypertrophied hearts. Using ion imaging technique, our results had obviously shown that the positive inotropic action of MCI-154 on hypertrophied myocytes was *via* sensitization as on normal cardiac myocytes did.

There is little information on the effects of MCI-154 on myocardial relaxation in pathologic hearts. Theoretically, calcium sensitizers should be disadvantageous to relaxation since the left shifting of the relationship between Ca^{2+} concentration and force. In fact, our study suggested that MCI-154 did not impair but slightly improve diastolic function assessed by TR_{50} and TR_{90} in isolated myocytes and $-dp/dt_{max}$ in isolated hearts though no statistical significance. One of the possible mechanisms accounted for this result is the selective Ca^{2+} sensitization. Liao, *et al*[21], by measuring Mg-ATPase activity of failing human my-

ocardium, showed that MCI-154 produced Ca^{2+}-sensitizing effect only at the higher systolic Ca^{2+} range, and it decreased Mg-ATPase activity at the diastolic Ca^{2+} range. They further revealed that the selective Ca^{2+} sensitization by MCI-154 at the higher systolic Ca^{2+} range may be due to the Ca^{2+}-induced exposure of hydrophobic patches on tropic C that facilitates the interaction of MCI-154 to contractile proteins.

In summary, the present results suggest that ① MCI-154 increases cardiac systolic function of hypertrophied rat hearts; ② MCI-154 enhance the positive inotropic effect in hypertrophied myocytes *via* Ca^{2+} sensitization; ③ MCI-154 do not impair the diastolic function of hypertrophied rat hearts.

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MCI-154 对心肌肥厚大鼠离体心脏的正性变力作用及其机制

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摘要：目的　探讨钙增敏剂 MCI-154〔6-[4-(4-吡啶氨基)苯基]-4,5-二氢-3(2*H*)哒嗪酮〕对心肌肥厚心脏与对正常心脏的作用是否不同及有关的机制。**方法**　利用离体心脏灌流技术观察 MCI-154 对心肌肥厚大鼠心功能的影响;应用离子影像学分析系统同步测定心肌细胞钙浓度瞬变和细胞长度。**结果**　① MCI-154　100 ~ 400 μmol·L^{-1}范围内浓度依赖性地提高了心肌肥厚大鼠心功能的各项指标。在 400 μmol·L^{-1}时,左室主动收缩压(左室收缩峰压与左室舒张末压之差)及左室压最大上升速率(+ dp/dt_{max})与对照值相比显著增加,左室压最大下降速率(– dp/dt_{max})有增高趋势,但无统计学意义;② MCI-154 10 ~ 100 μmol·L^{-1}在钙瞬变无明显改变情况下,呈浓度依赖性增加肥厚心肌细胞的缩短程度和钙敏感性; ③ MCI-154 对肥厚心肌细胞钙瞬变的 50% 和 90% 恢复时间影响不大。**结论**　在心肌肥厚大鼠心脏上,和在正常大鼠心脏上一样,MCI-154 主要通过钙增敏作用发挥其正性变力作用。

关键词： 钙增敏剂; MCI-154; 心脏肥厚; 心脏收缩; 钙, 细胞内

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