

## Effect of MCI-154, a calcium sensitizer, on calcium sensitivity of myocardial contractile system in endotoxemic rats<sup>1</sup>

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**KEY WORDS** endotoxemia; cardiotoxic agents; papillary muscles; calcium; MCI-154

### ABSTRACT

**AIM:** To investigate the effect of MCI-154 on calcium sensitivity of myocardial contractile system in endotoxemic rats. **METHODS:** Skinned right ventricular papillary muscles from endotoxemic rats were prepared by saponin 500 mg/L. Forces of the skinned muscles were recorded when they were activated sequentially by different concentrations of  $Ca^{2+}$  with or without cardiotoxic agents. The tension-pCa relationship and pCa<sub>50</sub> of the skinned fibers were taken as the index of  $Ca^{2+}$  sensitivity of myocardial contractile system. **RESULTS:** The maximal  $Ca^{2+}$ -activated tension ( $T_{max}$ ) was lower, and pCa<sub>50</sub> was reduced in endotoxemia group as compared with those in sham control group. Milrinone 50  $\mu$ mol/L could not counteract the above abnormalities. However, after MCI-154 10  $\mu$ mol/L was added, the  $T_{max}$  and pCa<sub>50</sub> were increased to an extent similar to that of sham control group and significantly higher than those of endotoxemia group and endotoxemia + milrinone group. Furthermore, such effects of MCI-154 were concentration-dependent. **CONCLUSION:** The  $Ca^{2+}$  sensitivity of cardiac contractile system in endotoxemic rats is decreased. MCI-154 can reverse the decreased sensitivity and increase  $T_{max}$  of myocardial muscles from endotoxemic rats.

### INTRODUCTION

Evidence suggests that a decrease in  $Ca^{2+}$  sensitivity of myocardial contractile proteins is one of the important causes of cardiac depression during endotoxic shock<sup>[1]</sup>. Recently, a new kind of positive inotropic agents,  $Ca^{2+}$  sensitizers, have been developed. It has been shown that  $Ca^{2+}$  sensitizers can augment myocardial contractility and improve cardiac function by interacting with troponin C (TnC), increasing the affinity of TnC for  $Ca^{2+}$ <sup>[2, 3]</sup> and prolonging the life span of the  $Ca^{2+}$ -induced conformation of TnC<sup>[4]</sup>. In order to improve the cardiac depression during endotoxic shock, it seems to be more desirable to restore the initial high  $Ca^{2+}$  sensitivity by means of a calcium sensitizer, rather than to increase the intracellular free  $Ca^{2+}$  concentration ( $[Ca^{2+}]_i$ ) that is likely to cause  $Ca^{2+}$  overload in myocytes. As yet, however, little information is available about the effect of a calcium sensitizer on  $Ca^{2+}$  sensitivity of myocardial contractile proteins in endotoxic shock. We previously reported that MCI-154, {6-[4-(4'-pyridyl)aminophenyl]-4,5-dihydro-3(2H)-pyridazinone hydrochloride}, a calcium sensitizer, can produce remarkable therapeutic effects on endotoxic rabbits<sup>[5]</sup>, and that MCI-154 can augment the contractility of the left ventricular papillary muscles from endotoxic rats<sup>[6]</sup>. Thus, it was of interest to explore the possible mechanism underlying the above effects of MCI-154. In this study, we investigated the effect of MCI-154 on  $Ca^{2+}$  sensitivity of myocardial contractile proteins using skinned fibers from the right ventricular papillary muscles of endotoxemic rats.

### MATERIALS AND METHODS

#### Models of endotoxic shock and sham shock

Thirty-two male and female (1:1) Wistar rats (120-150 g, Grade I, Certificate No 19050) were provided by Experimental Animal Institute of Third Military University. The rats were fasted for 12 h prior to the experiment but were allowed water *ad lib* and were randomly divided in-

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to four groups of eight each: group I, sham control group; group II, endotoxemic group; group III, endotoxemia + milrinone 50  $\mu\text{mol/L}$  group; group IV, endotoxemic + MCI-154 10  $\mu\text{mol/L}$  group. Endotoxemia or sham control was induced by ip injection of endotoxin 10  $\text{mg}\cdot\text{kg}^{-1}$  or equivalent sterile saline, respectively. Eight hours after the injection of endotoxin and saline, the models were completed<sup>[7]</sup>. The percentage fall in the mean arterial pressure (MAP) was 40 % - 50 % at 8 h following the injection of endotoxin.

**Preparations of skinned right ventricular fibers** Skinned right ventricular papillary muscles of rats were prepared according to the method of Huang *et al*<sup>[8]</sup> with some modifications. Eight hours after the injection of endotoxin or saline, the rat was decapitated and the heart was rapidly excised and placed in an oxygenated physiological solution of the following composition (mmol/L): NaCl 130, KCl 4,  $\text{CaCl}_2$  1.5,  $\text{MgCl}_2$  0.5, glucose 10, Tris 10, bubbled with 95 %  $\text{O}_2$  + 5 %  $\text{CO}_2$ , pH 7.4, at 25 °C. A small bundle of papillary muscles with a diameter < 1 mm was dissected from the right ventricle and mounted in a tissue bath containing 20 mL relaxing solution between two miniature clamps. The composition of the solution was as follows (mmol/L): KCl 140,  $\text{MgCl}_2$  5, egtazic acid 10, imidazole 25,  $\text{Na}_2\text{ATP}$  5, phosphocreatine (PC) 10, phosphocreatine kinase (PCK) 0.1 g/L, pH 7.2, at 25 °C. The upper clamp was connected by silk suture to a precalibrated force transducer (Statham UC2, Gould, USA) for measuring isometric tensions on a multichannel recorder (RM6200, Nihon Khoden, Japan). The lower clamp was mounted directly on a stationary rod at the bottom of the tissue bath. The muscles were equilibrated for 15 min. To obtain skinned fibers, the muscles were exposed to skinning solution (relaxing solution containing saponin 500 mg/L) at 25 °C for 30 min. Saponin 500 mg/L perforated not only the surface membrane but also the membrane of sarcoplasmic reticulum (SR)<sup>[9]</sup>. Then, the skinning solution was washed out completely with relaxing solution, and muscles were stretched to 130 % of their natural length (length without resting tension in the bath) with an integral micromanipulator.

**Determination of  $\text{Ca}^{2+}$  sensitivity of the skinned fibers** Force induced in the skinned papillary fibers was recorded when they were activated sequentially by different  $\text{Ca}^{2+}$  activating solutions. The concentration of free  $\text{Ca}^{2+}$  was expressed as pCa ( $-\lg[\text{Ca}^{2+}]$ ).  $\text{CaCl}_2$  and egtazic acid were added to make different acti-

vating solutions with pCa ranging from 7.0 - 4.0 ( $\text{CaCl}_2$  3.83 - 10.31 mmol/L), pH 7.2, at 25 °C according to the program developed by Fabiato *et al*<sup>[10]</sup> (supplied by Laboratory of Cardiovascular Physiology, Hunan Medical University, China). In group III and group IV, the skinned fibers from endotoxemic rats were activated by different pCa activating solutions containing milrinone 50  $\mu\text{mol/L}$  or MCI-154 10  $\mu\text{mol/L}$ , respectively (our preliminary experimental result; the  $\text{EC}_{50}$  of MCI-154 was 5 times less than that of milrinone). The activating time for each experiment was 5 min. At the end of the experiment, the muscles were dried by filter papers and weighed. The  $\text{Ca}^{2+}$ -activated tensions were normalized by muscle's cross-sectional area (CSA), which was calculated by dividing muscle mass by specific gravity and the length of the muscle. The tension recorded at pCa 4.0 was taken as the maximum ( $T_{\text{max}}$ ). Relative tension at pCa 7.0 - 4.5 was expressed as the percent of  $T_{\text{max}}$ . Tension-pCa relationship curves were plotted, and pCa<sub>50</sub> was calculated using Hill equation:  $T = [\text{Ca}^{2+}]^n / (K^n + [\text{Ca}^{2+}]^n)$ , where  $T$  is relative tension (%),  $n$  is the Hill coefficient, and  $K$  is  $\text{Ca}^{2+}$  concentration required for producing 50 % of  $T_{\text{max}}$ . ( $\lg K$  is the curve's medium value pCa<sub>50</sub>, which can be taken as the quantitative index of the affinity of TnC for  $\text{Ca}^{2+}$ <sup>[8]</sup>).

**Effect of different concentrations of MCI-154 on  $\text{Ca}^{2+}$  sensitivity of skinned right ventricular papillary muscles** Three right ventricular papillary muscles isolated from each rat in group IV were used in this experiment. Muscles were divided into 3 groups with activating solutions containing MCI-154 1  $\mu\text{mol/L}$  (group A,  $n=8$ ), 10  $\mu\text{mol/L}$  (group B,  $n=8$ ), 100  $\mu\text{mol/L}$  (group C,  $n=8$ ), respectively. The experimental procedures and data management were the same as that mentioned above.

**Drugs** Endotoxin (*Escherichia Coli*, O111:B4), saponin, PC, PCK,  $\text{CaCl}_2$ , egtazic acid,  $\text{Na}_2\text{ATP}$  were all purchased from Sigma Company. MCI-154 (grey powder,  $M_r$  266, mp 250 - 251 °C, Purity > 99.5 %) was supplied by College of Pharmacy, the Second Military Medical University, China; Milrinone was purchased from Chongqing Medical Industrial Company. All other chemicals were of analytical purity. All the solutions were prepared demineralized water. PC, PCK were added to the solutions shortly before the experiment. This study was performed in accordance to the Chongqing Council for Animal Research Guidelines for the Use of Experimental Animals.

**Statistical analysis** Experimental values were expressed as  $\bar{x} \pm s$ . Statistical differences between groups were assessed by analysis of variance and a multiple comparison procedure (Student-Newman-Keuls' test).

## RESULTS

**Effect of MCI-154 on  $Ca^{2+}$  sensitivity of the skinned fibers** There were no significant differences among the four groups with regard to the weight, length and CSA of the papillary muscles. The  $T_{max}$  in endotoxemia + MCI-154 group was increased to the level of sham control group, higher than those of endotoxemia group and endotoxemia + milrinone group ( $P < 0.05$ ) (Tab 1). The values of relative tensions at pCa 6.5–5.0 in endotoxemic group and endotoxemia + milrinone group were lower ( $P < 0.05$ ,  $P < 0.01$ , respectively), resulting in the rightward shift of the tension-pCa relationship curves (Fig 1). However, the relative tension at pCa 6.5–5.0 in endotoxemia + MCI-154 group were increased to an extent similar to that of sham control group, higher than those of endotoxemia group and endotoxemia + milrinone group ( $P < 0.05$ ,  $P < 0.01$ , respectively), and the tension-pCa relationship curves were shifted leftwards.

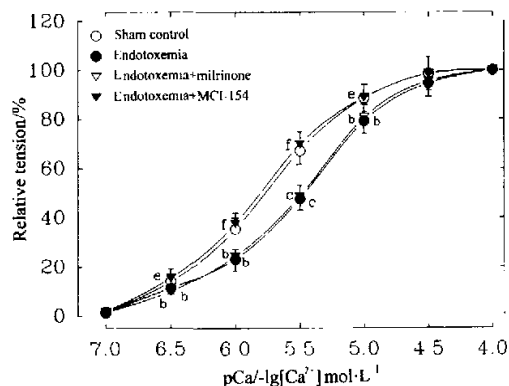


Fig 1. Change in relative tension of skinned papillary muscles from endotoxemic rats following administration of milrinone and MCI-154.  $n = 8$ ,  $\bar{x} \pm s$   $^bP < 0.05$ ,  $^cP < 0.01$  vs sham control.  $^eP < 0.05$ ,  $^fP < 0.01$  vs endotoxemia group.

The  $pCa_{50}$  in the four groups are shown in Tab 1. The values of  $pCa_{50}$  in endotoxemia group and endotoxemia + milrinone group were decreased, 0.35 and 0.33 pCa units, respectively as compared with that of sham

control group ( $P < 0.05$ ). On the contrary, the  $pCa_{50}$  in endotoxemia + MCI-154 group was increased compared with endotoxemia group and endotoxemia + milrinone group ( $P < 0.05$ ). This value was a little higher than the  $pCa_{50}$  of sham control group but with no statistical difference.

**Concentration-response of MCI-154** The values of  $T_{max}$  ( $10^{-3} \times T_{max}/N \cdot m^{-2}$ ) in three groups were: 1  $\mu\text{mol/L}$  group,  $19.63 \pm 1.71$ ; 10  $\mu\text{mol/L}$  group,  $21.30 \pm 1.77$ ; 100  $\mu\text{mol/L}$  group,  $21.78 \pm 1.93$ . The  $T_{max}$  in the latter two groups were higher than that in 1  $\mu\text{mol/L}$  group ( $P < 0.05$ ). As the concentration of MCI-154 increased, the relative tensions at pCa 6.5–5.0 increased ( $P < 0.05$ ,  $P < 0.01$ , respectively), resulting in further leftward shift of tension-pCa relationship curves (Fig 2). The  $pCa_{50}$  in 3 groups was: 1  $\mu\text{mol/L}$  group,  $5.72(5.44 - 6.00)$ ; 10  $\mu\text{mol/L}$  group,  $5.84(5.65 - 6.03)$ ; 100  $\mu\text{mol/L}$  group,  $6.12(5.91 - 6.33)$ . The  $pCa_{50}$  MCI-154 100  $\mu\text{mol/L}$  group was significantly higher than those in the other two groups ( $P < 0.05$ ).

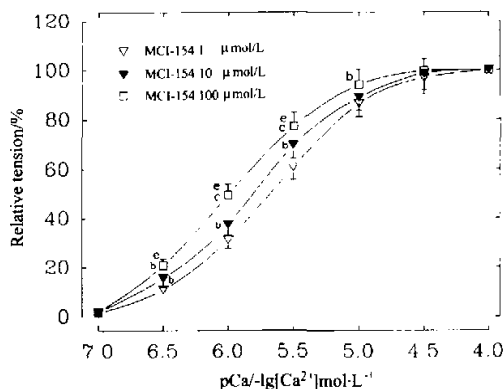


Fig 2. Effect of different concentrations of MCI-154 on relative tension of skinned papillary muscles from endotoxemic rats.  $n = 8$ ,  $\bar{x} \pm s$ .  $^bP < 0.05$ ,  $^cP < 0.01$  vs MCI-154 1  $\mu\text{mol/L}$ .  $^eP < 0.05$  vs MCI-154 10  $\mu\text{mol/L}$ .

## DISCUSSION

Although studies conducted in isolated myocardial tissues and isolated myocytes from endotoxic shock animals showed that  $Ca^{2+}$  sensitivity of myocardial contractile system was decreased<sup>[1,11]</sup>, the mechanisms responsible for the phenomenon remain obscure. Experiments have suggested that the decreased  $Ca^{2+}$  sensitivity of

**Tab 1. Effects of MCI-154 on maximal activated tension ( $T_{max}$ ) and  $Ca^{2+}$  sensitivity of skinned right ventricular papillary muscles in endotoxemic rats. I: sham control; II: endotoxemia; III: endotoxemia + milrinone; IV: endotoxemia + MCI-154.  $n = 8$ ,  $\bar{x} \pm s$  for  $T_{max}$ .  $^bP < 0.05$  vs I.  $^cP < 0.05$  vs II.**

	Group			
	I	II	III	IV
$10^{-3} \times T_{max}/N \cdot m^{-2}$	$22.12 \pm 1.62$	$18.47 \pm 1.70^b$	$19.01 \pm 2.18^b$	$21.30 \pm 1.77^c$
$pCa_{50}$	$5.82(5.64 - 6.00)$	$5.47(5.31 - 5.63)^b$	$5.49(5.28 - 5.70)^b$	$5.84(5.65 - 6.03)^c$

myocardial filaments during hypoxia, ischemia was associated with changes in the levels of intramyocardial metabolites<sup>[12,13,14]</sup>, eg, the decrease in pH<sup>[12,14]</sup>, and the decline in PC concentration or accumulation of inorganic phosphate (Pi) near crossbridges<sup>[13]</sup>. In fact, studies showed that acidosis and the addition of Pi decreased  $Ca^{2+}$  sensitivity of the contractile proteins in normal cardiac fibers and reconstituted thin filaments<sup>[15,16]</sup>. Possibly, the above abnormalities exist and contribute to the decreased  $Ca^{2+}$  sensitivity of myocardial contractile system during endotoxic shock. Therefore, it is of clinical significance to survey novel cardiotoxic agents that improve the cardiac depression during endotoxic shock by increasing  $Ca^{2+}$  sensitivity of myocardial contractile system. Our previous study showed that MCI-154, a calcium sensitizer, could produce a strong positive inotropic effect on myocardium from endotoxic shock rats<sup>[6]</sup>. But the mechanisms underlying the effect are unclear.

By using the skinned myocardial preparations, we investigated the effect of MCI-154 on  $Ca^{2+}$  sensitivity of the myocardial contractile system in endotoxemic rats. Our results indicated that the  $Ca^{2+}$  sensitivity in endotoxemic rats was indeed decreased, as reflected by the decrease of  $T_{max}$  and  $pCa_{50}$ . Milrinone, a phosphodiesterase (PDE) inhibitor, could not increase the  $T_{max}$  and  $pCa_{50}$  of the skinned papillary fibers from endotoxemic rats. However, when skinned right ventricular papillary muscles were treated with activating solution containing MCI-154, the  $T_{max}$  was augmented to an extent similar to that of sham control group; the values of relative tensions at  $pCa$  6.5 - 5.0 were markedly increased with a right shift in the tension- $pCa$  relationship. The  $pCa_{50}$  in MCI-154 treated group was significantly higher than that in endotoxemia group and endotoxemia + milrinone group. Furthermore, the above effects of MCI-154 were concentration-dependent.

The leftward shift in tension- $pCa$  relationship and the increase of  $pCa_{50}$  in this study suggested that the

affinity of TnC for  $Ca^{2+}$  was enhanced by MCI-154. The increase of  $T_{max}$  indicated that MCI-154 could directly facilitate the interaction between actin and myosin and increase the ATPase activity of myocardial contractile proteins in endotoxemic rats. The present study is in agreement with the experiment conducted by Kitada *et al*<sup>[15]</sup>, which showed that MCI-154 increased the maximum tension,  $pCa_{50}$  of the skinned fibers from myocardial papillary muscles of normal guinea pigs. So, the mechanisms of effects of MCI-154 found in normal myocardial tissue may also play important roles in increasing the  $Ca^{2+}$  sensitivity of myocardial contractile system in endotoxemic rats. Considering that a decrease in  $Ca^{2+}$  sensitivity of myocardial contractile apparatus<sup>[1]</sup> and  $Ca^{2+}$  overload in myocytes<sup>[17]</sup> contributed to the loss of cardiac contractility during endotoxic shock, MCI-154 may have advantages over conventional cardiotoxic agents for improving cardiac dysfunction under this condition.

In conclusion, the present study has revealed that MCI-154 can directly increase the  $Ca^{2+}$  sensitivity of the contractile proteins in the skinned myocardial fibers from endotoxemic rats. This may be responsible for its therapeutic actions in the treatment of endotoxic shock.

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### 钙增敏剂 MCI-154 对内毒素血症大鼠心肌收缩系统钙敏感性的影响<sup>1</sup>

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**关键词** 内毒素血症; 强心药; 乳头状肌; 钙; MCI-154

**目的:** 研究 MCI-154 对内毒素血症大鼠心肌收缩系统钙敏感性的影响。 **方法:** 利用皂素 500 mg/L 制备内毒素血症大鼠脱膜右心室乳头状肌标本, 用不同钙浓度的含或不含强心药物的激活液进行顺序激活, 记录 Ca<sup>2+</sup> 激活张力。 **结果:** 同正常对照组相比, 内毒素血症大鼠脱膜乳头状肌钙最大激活张力 (T<sub>max</sub>) 降低, pCa<sub>50</sub> 值下降。 甲腈吡酮 50 μmol/L 对上述异常无明显的改善作用。 内毒素血症大鼠脱膜乳头状肌经含 MCI-154 10 μmol/L 的激活液处理后, T<sub>max</sub> 和 pCa<sub>50</sub> 值明显增加, 与假休克组类似, 明显高于内毒素血症组和内毒素血症 + 甲腈吡酮组值。 MCI-154 的上述作用还具有剂量依赖性。 **结论:** 大鼠内毒素血症后, 心肌收缩系统对 Ca<sup>2+</sup> 敏感性降低, MCI-154 可明显增加内毒素血症大鼠心肌收缩蛋白对钙的敏感性, 增加心肌钙最大激活张力。

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