利用度．结果显示：微粉型和普通型 Gli 的 $C_{max}$ 分别为 529±73 ng·ml$^{-1}$ 和 212±41 ng·ml$^{-1}$；$T_{max}$ 前者为 2.2±0.3 h，后者为 3.5±0.6 h．微粉型 Gli 的相对生物利用度(F)约为 77%．两种剂型的其它药动学参数，如 $T_{\frac{1}{2}}$，$V_d$，$Cl$ 等无显著性差异．

关键词 格列苯脲；药物动力学；剂型；高压液相色谱法；人类实验

药代动力学

BIBLID： ISSN 0253-9756 中国药理学报 Acta Pharmacologica Sinica 1993 May；14 (3)：197—200

# Effects of lisinopril and captopril on calcium in rat heart

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**ABSTRACT** We studied the effects of lisinopril (Lis) and captopril (Cap), two angiotensin-converting enzyme inhibitors, on calcium in ischemia/reperfusion and normal rat hearts. Ischemia/reperfusion hearts were subjected to 15 min ischemia followed by 1 or 30 min reperfusion. Lis 0.1 μmol·L$^{-1}$ and Cap 200 μmol·L$^{-1}$ decreased the concentration of calcium in ischemia/reperfusion hearts (the content of calcium in reperfusion 1 min heart were reduced from 4.0±0.6 to 2.7±0.5 and 3.0±0.9 μmol/g dry wt respectively). In cultured cell of neonatal rat heart, both drugs inhibited the uptake of $^{45}Ca^{2+}$. The activity of $Na^+$, $K^+$-ATPase prepared from rat heart was increased (activity increased from 15.7±2.3 in control group to 21.2±2.0 and 22.0± 3.1 μmol/h mg protein in Lis and Cap groups, respectively). This calcium lowering effects of Lis and Cap may be important in protecting the ischemia/reperfusion damage of myocardium.

**KEY WORDS** angiotensin-converting enzyme inhibitors; cultured cells; ventricular fibrillation; calcium radioisotopes; heart; myocardial reperfusion injury

Angiotensin-converting enzyme inhibitor (ACEI) have protecting effects against hypertension and heart failure clinically. Recently ACEI has been ascribed to decrease the size of myocardial infarction and alleviate the damage caused by ischemia/reperfusion in animal heart both *in vitro* and *in vivo*[1-3]. Our experiments have demonstrated that lisinopril (Lis) protected the myocardial ischemia/reperfusion injury in Langendorff rat heart[4,5]. Myocardial injury during ischemia/reperfusion was thought to be associated with massive accumulation of intracellular calcium and the increase of free radicals. In the present study, effects of Lis and Cap on calcium in the rat heart were studied.

Received 1991-11-11 Accepted 1992-08-12

## MATERIALS AND METHODS

Lis was a gift from Merck Sharp & Dohme Research Lab, NJ, USA; Cap was a product of Dandong Pharmaceutical Factory, China; $^{45}CaCl_2$ (999 MBq·g$^{-1}$) was purchased from China Institute of Atomic Energy, Beijing. Wistar rat of either sex weighing 250±$s$ 30 g were provided by our Academy.

**Effects on ventricular fibrillation (VF) in ischemia/reperfusion rat heart** Working heart and Langendorff hearts were perfused with buffer contained: NaCl 117.2, KCl 5.37, $MgSO_4 \cdot 7H_2O$ 0.8, $NaH_2PO_4 \cdot 2H_2O$ 0.92, $CaCl_2$ 2.47, $NaHCO_3$ 25, EDTA 0.05, glucose 11.1 (mmol·L$^{-1}$), which was equilibrated with 95% $O_2$+5% $CO_2$, pH 7.4, at 36.5 and 37.5℃. The perfusion buffer was filtered through a $G_3$ filter before reaching the heart. Preload and afterload were held at 1.17 and 6.93 kPa, on the work-

ing heart, respectively. Langendorff heart was perfused at 7 ml · $min^{-1}$, controlled with a piper peristaltic pump. Ischemia was induced by ligating the left descending branch of coronary artery for 15 min. Reperfusion started after the release of ligation and endured for 30 min. In the treated groups, ACEI were added to the buffer from the preligation time up to the end, and in control group, saline solution was added to. The VF was scrutinized by ECG.

**Measurement of $Ca^{2+}$ ion in ischemia/reperfusion rat heart** In Langendorff rat heart the left descending branch of coronary artery was ligated for 15 min and then reperfused for 1 or 30 min, respectively. At the end of reperfusion, the hearts were perfused with ice cold ion-free solution containing sucrose 0.35 mol · $L^{-1}$ and histidine 5 mmol · $L^{-1}$ to wash out the extracellular calcium[6,7]. The myocardium of left and right ventricles was dried to a constant weight and digested over 12 h in 5 ml mixed acid ($HNO_3$ 10 mol · $L^{-1}$ : $HClO_4$ 6 mol · $L^{-1}$, 20 : 1 ). The fluid after digestion was heated to dryness on an electric stove, and then the residue was dissolved in 5 ml of $HNO_3$ 0.5 mol · $L^{-1}$. Concentration of calcium was determined by an atomic absorption spectrophotometer (WYX-402 A, Shenyang, China).

***$^{45}Ca^{2+}$ uptake in cultured myocardial cells of neonatal rat*** Cardiomyocytes of 3 day old rat were seeded in scintillative counting vial at a concentration of $1 \times 10^6$ cells · $ml^{-1}$. Cells were grown in Eagle's medium containing 5% fetal calf serum at 37 C. The grown medium was replaced after 3 d by a fresh medium containing no fetal calf serum. At this stage, 0.1 ml $^{45}Ca^{2+}$ (contained $1.67 \times 10^4$ Bq) and ACEI were added to the medium. The cultures were incubated for 2 h at 37 C. The uptake was determined by removing the radioactive medium and washing 3 times at 25℃ with fresh medium containing no fetal calf serum (5 ml, each time). The washed cells were digested by a mixture of $HClO_4$ and $H_2O_2$ for 2 h at 70 C. The radioactivity was measured with a scintillation counter[8].

**Determination of $Na^+$, $K^+$-ATPase activity** Rats were decapitated and their hearts were excised[9]. $Na^+$,$K^+$-ATPase was prepared. The enzyme was incubated with ACEI. Enzymatic activity was assessed by method of determining phosphate[10].

## RESULTS

**Effects on VF** In rat working heart during ischemia/reperfusion, the incidence of VF were 100%. The incidence decreased significantly in groups treated with Lis (0.1 μmol · $L^{-1}$) and Cap (100 μmol · $L^{-1}$). In Langendorff rat heart, the incidences of VF were 81.25%, 23.18%, and 30.77% in ischemia/reperfusion, Lis, and Cap groups, respectively (Tab 1).

**Tab 1. Effects of ACEI on incidence of ventricular fibrillation (VF) in ischemia/reperfusion (I—R) rat hearts.**

| Group | μmol · $L^{-1}$ | $n$ | VF incidence | Rate, (%) |
|---|---|---|---|---|
| Working heart | | | | |
| Saline | | 10 | 10 | 100 |
| Captopril | 200 | 7 | 3 | 43 |
| | 20 | 5 | 3 | 60 |
| | 2 | 5 | 4 | 80 |
| Lisinopril | 0.1 | 7 | 2 | 29 |
| | 0.01 | 5 | 3 | 60 |
| | 0.001 | 5 | 5 | 100 |
| Langendorff heart | | | | |
| Saline | | 16 | 13 | 81 |
| Captopril | 200 | 13 | 3 | 23 |
| Lisinopril | 0.1 | 13 | 4 | 31 |

**Effects on $Ca^{2-}$ in Langendorff rat heart** After reperfusion for 1 min, the content of $Ca^{2+}$ in rat heart was increased. Both Lis and Cap were shown to lower the content of $Ca^{2+}$. When reperfused for 30 min, Cap also reduced the content of $Ca^{2+}$ (Tab 2).

**Effects of on $^{45}Ca^{2-}$ uptake in cultured cardiomyocytes** Uptake of $^{45}Ca^{2+}$ could be separated into 2 phases; a rapid phase and a slow one. We studied the slow phase, completed in 120 min. To wash away the extracellular $^{45}Ca^{2+}$, the effects of ACEIs on $^{45}Ca^{2+}$ uptake were measured by intracellular $^{45}Ca^{2+}$ cpm. In control group, the $^{45}Ca^{2+}$ content

**Tab 2. Effects of ACEI on $Ca^{2+}$ content (μmol/g dry wt) in Langendorff rat hearts. $n=7$ except Lis $n=6$. $\bar{x}\pm s$, $^{+}P>0.05$, $^{++}P<0.05$ *vs* normal; $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* ischemia/reperfusion (I—R).**

| | μmol·L$^{-1}$ | Reperfusion 1 min | Reperfusion 30 min |
|---|---|---|---|
| Normal | | 3.2±0.5 | 3.3±0.6 |
| I—R | | 4.0±0.6$^{++}$ | 4.4±1.1$^{++}$ |
| Captopril | 200 | 3.0±0.9$^{**}$ | 3.1±0.4$^{**}$ |
| Lisinopril | 0.1 | 2.7±0.5$^{***}$ | 3.4±0.4 |

was 1.17 ± 0.23 pmol/1 × $10^6$ cell. After treated with Lis 0.1 μmol·L$^{-1}$ and Cap 200 μmol·L$^{-1}$, the content of $^{45}Ca^{2+}$ in culture cardiomyocytes were reduced to 0.69±0.12 and 0.60±0.18 pmol / 1 × $10^6$ cell, respectively. The data showed that Lis and Cap inhibited the uptake of $^{45}Ca^{2+}$ siginificantly.

**Effects on $Na^+$, $K^+$-ATPase activity in rat heart** Lis (0.1-0.01 μmol·L$^{-1}$) elevated the enzyme activity remarkably. Cap 200 μmol·L$^{-1}$ produced the same effect, but not at 20 μmol·L$^{-1}$. An increase of enzyme activity would result in a decrease of intracellular calcium (Tab 3).

**Tab 3. Effects of ACEI on $Na^+$, $K^+$—ATPase activity in rat hearts. $\bar{x}\pm s$, $^{*}P>0.05$, $^{***}P<0.01$ *vs* saline.**

| Group | μmol·L$^{-1}$ | $n$ | Enzyme activity μmol/h·mg protein |
|---|---|---|---|
| Saline | | 12 | 15.7±2.3 |
| Captopril | 20 | 8 | 16.5±2.9 |
| Captopril | 200 | 8 | 22.0±3.1$^{***}$ |
| Lisinopril | 0.1 | 8 | 21.2±2.0$^{***}$ |
| Lisinopril | 0.01 | 8 | 21.4±4.0$^{***}$ |

## DISCUSSION

We used working heart and Langendorff heart in our experiments and found that Lis and Cap significantly lowered the incidence of ventricular fibrillation induced by ischemia/reperfusion in both heart preparations. Ventricular fibrillation occurred as a consequence of serious ischemia/reperfusion injury of the heart when the cardiac rhythm and automaticity were handicapped by the injury. Our experiments suggested that Lis and Cap possessed an protective effect against ischemia/reperfusion injury.

Overload calcium is one of the main factors causing myocardial damage in ischemia/reperfusion. When reperfused, the concentration of intracellular calcium would hardly be enhanced. Lis and Cap both lowered the concentration of calcium in reperfusion 1 min in Langendorff rat hearts. The uptake of $^{45}Ca^{2+}$ by cultured cardiomyocytes could be resolved into 2 phases. The initial phase occurred between 1 s and 1 min. Then there was a subsequent slower uptake, completed in 120 min. In the slower phase, the calcium influx occurred via the slow calcium channel and other pathways, eg. $Na^+$-$Ca^{2+}$ exchange[11]. Lis and Cap decreased the concentration of intracellular $^{45}Ca^{2+}$. Otherwise, both ACEIs enhanced the activity of $Na^+$,$K^+$-ATPase. The activities provided more evidence to the reduction of overloaded intracellular calcium. In conclusion, the lowering of concentration of calcium in rat heart might be one of the important mechanisms eliciting protection against ischemia/reperfusion damage.

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## 赖诺普利和卡托普利对大鼠心脏钙的影响

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**摘要** 本文报道了血管紧张素转换酶抑制剂对大鼠心肌的影响. 赖诺普利(Lis) 0.1 μmol·$L^{-1}$和卡托普利(Cap) 200 μmol·$L^{-1}$减少缺血再灌大鼠心肌钙的浓度，降低培养新生大鼠心肌细胞内$^{45}Ca^{2+}$的含量，提高心肌 $Na^+$,$K^+$—ATPase 的活性. 结果提示减少钙的浓度可能是 Lis 和 Cap 抗大鼠心肌缺血再灌损伤的重要因素之一.

**关键词** 血管紧张素转换酶抑制剂；培养细胞；心室纤颤；钙放射性同位素；心脏；心肌再灌注损伤

钙

BIBLID : ISSN 0253-9756 中国药理学报 *Acta Pharmacologica Sinica* 1993 May; 14 (3) : 200—202

# Plasma bevantolol concentration and heart rate in rabbits

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**ABSTRACT** Bevantolol (Bev, 5, 10 mg·$kg^{-1}$) was injected iv to rabbits. A measure the lag time of heart rate (HR) response behind the changes in plasma Bev concentrantion ($K_{eo}$), and the sensitivity of the site of action of Bev ($EC_{50}$) were determined. The $K_{eo}$ were 0.03±0.02 and 0.029±0.009 $min^{-1}$ and the $EC_{50}$ were 0.2±0.1 and 0.27±0.14 μg·$ml^{-1}$ respectively for the 2 dosages. The peak changes in HR lagged behind the changes in plasma Bev concentration. There were no significant changes in both pharmacokinetic and pharmacodynamic parameters between the 2 dosages.

**KEY WORDS** bevantolol; pharmacokinetics; heart rate

Bevantolol (Bev), a cardioselective adrenergic beta receptor blockader has no intrinsic sympathomimetic activity and has weak membrane stabilizing and local anesthetic properties[1]. Bev is effective in the treatment of angina[2] and hypertension[3] in humans and shows anti-arrhythmic and anti-ischemic

Received 1992-04-21 Accepted 1992-12-24