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Calcium Antagonists Depress Cardiac Function after Coronary Ligation in Rats.

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Abstract

Calcium antagonists are currently approved for use in patients with arrhythmias, stable and unstable angina pectoris, and systemic hypertension. However, the effects of calcium antagonists upon the outcome of experimental or clinical myocardial infarction are still debated. We evaluated the effects of calcium antagonists on the isolated perfused rat heart which coronary was ligated. Forty four isolated rat heart-lung preparations from male Wistar rats weighing 300–320 g were randomly divided into 4 groups according to the used drug as follows:

1. Control (C) group; no drug. 2. Diltiazem (D) group; $4x10^{-7}$ M of diltiazem. 3. Nicardipne (N) group; 100 ng/ml of nicardipine. 4. Verapamil (V) group; $3x10^{-7}$M of verapamil. The left anterior descending coronary artery was ligated and one of calcium antagonists was administered 8 min and 13 min after the start of perfusion, respectively. Thirty min after the perfusion, the heart was divided into infarcted and non-infarcted regions and freeze-dried for 6 days. Myocardial high energy phosphates (ATP, ADP and AMP) were measured by the high performance liquid chromatography. Myocardial lactate (L) and pyruvate (P) were measured by the enzymatic method. Coronary ligation did not influence hemodynamics in all groups, but verapamil decreased cardiac output and left ventricular (LV) dP/dt max significantly in the coronary ligated heart. There was no significant difference in the weights of infarcted regions produced by ligation among the groups. However, the weight of infarcted region in the V group was heavier than that of non-infarcted region. In all groups, myocardial ATP levels in infarcted regions were significantly lower than those in non-infarcted regions. In addition, myocardial ADP, AMP levels and L/P ratio in infarcted regions were significantly higher than those in non-infarcted regions. However, there were no significant differences in high energy phosphates levels and L/P ratio among the groups. Although all calcium antagonists did not influence the metabolism in the infarcted heart, they, especially verapamil, caused LV depression. This may support the clinical impression that most of calcium antagonists available are not clearly safe in patients with myocardial infarction.

Key words : Calcium antagonists, Myocardial infarction, Myocardial metabolism.

Introduction

Calcium antagonists are currently approved for use in patients with arrhythmias, stable and unstable angina pectoris, and systemic hypertension. Many animal data had indicated a potential for benefit from calcium antagonists for ischemic heart due to a reduction of afterload, improved coronary perfusion and a direct myocardial effect[1~3]. However, recent reviews of trials on the role of calcium antagonists as treatment for myocardial infarction in the immediate postinfarction period did not provide evidence of

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efficacy[4,5]. This discrepancy has been still debated. Therefore, the present study was designed to evaluate the effects of calcium antagonists for cardiac function and ischemic size on the isolated perfused rat heart when its coronary was acutely ligated. This experiment explores the question of whether the choice of calcium antagonists can influence the degree of myocardial ischemia after coronary ligation.

Materials and Methods

This study was approved by the animal care committee of Yamanashi Medical University. The techniques used were almost identical to those used in an earlier study[6]. Briefly, 44 male Wistar-ST rats (300–320 g) were randomly divided into 4 groups according to given drugs as follows. 1. Control (C) group; no drug. 2. Diltizem (D) group; $4x10^{-7}$ M of diltiazem. 3. Nicardipne (N) group; 100 ng/ml of nicardipine. 4. Verapamil (V) group; $3x10^{-7}$ M of verapamil. These were comparable to therapeutic serum concentrations[7~9]. All rats were anesthetized with isoflurane only during the preparation. After tracheostomy trachea was intubated and intermittent positive pressure ventilation was adjusted to maintain $PaCO_2$ at 4.7–5.3 kPa and PaO_2 at 40–53 kPa with a 95 %O_2+5 %CO_2 gas mixture. The chest was opened and flooded with ice-cold saline, and the heart arrested. Cannulae were inserted into the aorta and the superior and inferior venae cavae. The cannula in the superior vena cava was used for the monitor of right atrial pressure.

The heart lung preparation was perfused with a solution (25 ml) containing red blood cells collected from another rat and Krebs Ringer bicarbonate buffer solution, with hematocrit and pH of 25 % and 7.4, respectively. The concentrations (mM) of the buffer constituents were: NaCl 127, KCl 5.1, $CaCl_2$ 2.2, KH_2PO_4 1.3, $MgSO_4$ 2.6, $NaHCO_3$ 15, glucose 5.5 and heparin. The perfusate pumped from the heart passed through a pneumatic resistance and was collected in a reservoir kept at 37 ℃, and then returned to the inferior vena cava. In this model, since no other organs except heart and lung were perfused, cardiac output (CO) was determined by the inflow as long as the heart did not fail, and mean arterial pressure was regulated by the pneumatic resistance.

Heart rate (HR) was recorded with ECG through a bioelectric amplifier (AB-621G, Nihonkohden, Japan) and CO was measured with an electromagnetic blood flow meter (MFV-1200, Nihonkohden, Japan). Arterial pressure (AP) and right atrial pressure (RAP) were measured with transducers (TP101T and LPU-0.1A) and carrier amplifiers (AP-601G, Nihonkohden, Japan). The rates of left ventricular tension development (LV dP/dt) were calculated from aortic dP/dt obtained electronically[10].

After the preparation was completed, the heart was perfused initially with CO of 30 ml/min and mean AP of 70 mmHg by regulating the venous return and pneumatic resistance, respectively. After this through the study, the pneumatic resistance was kept constant. Eight min after the start of perfusion, the left anterior descending (LAD) coronary artery was ligated by a string. The site of ligation of the LAD coronary artery located approximately 3 mm from the aortic root. Thirteen min after the start of perfusion, one of the calcium antagonists was administered into the reservoir except in the C group. When RAP exceeded 7 cmH_2O, we reduced the venous return. Thirty min after the start of the perfusion, the heart was visibly divided into infarcted and non-infarcted regions, then immediately freeze-dried by liquid nitrogen for 6 days. The weights of infarcted and non-infarcted regions were measured and an aliquot was extracted with perchloric acid and centrifuged at 3000 rpm. Myocardial high energy phosphates(ATP, ADP and AMP) were measured by the high liquid performance chromatography[11] and the values were expressed as micromoles per gram of dry weight. Tissue lactate and pyruvate levels were determined spectrophotometrically by standard techniques[12].

Hemodynamic data within groups were analyzed by two way analysis of variance with repeated measures. The other data were analyzed by one way analysis of variance followed by the Fisher's PLSD test for multiple comparisons. A probability of $P<0.05$ was regarded as statistically significant. The data were given as mean±S.E.

Results

All hearts had no arrhythmias after the coronary ligation, such as ventricular fibrillation or A-V block. There were no significant differences in HR, systolic blood pressure (SBP) and RAP among the groups through the experiment. However, verapamil decreased CO (vs, values at 5 min) and LV dP/dt max (vs, control) significantly in the coronary ligated heart. Diltiazem and nicardipine also reduced LV dP/dt max significantly only at the end of perfusion (at 30 min) when compared with control (Table 1). There were no significant differences in the weights of infarcted regions and the ratio of infarcted and non-infarcted regions produced by ligation among the group. However, the weight of infarcted region in the V group was significantly heavier than that of non-infarcted region (Fig. 1). In all groups, myocardial ATP levels in infarcted regions were significantly lower than those in non-infarcted regions. In addition, myocardial ADP, AMP levels and L/P ratio in infarcted regions were significantly higher than those in non-infarcted regions. However, these were no significant differences in high energy phosphates and L/P ratio among the group (Fig. 2, 3).

Discussion

We have previously reported that 200 ng/ml of nicardipine and 450 ng/ml of diltiazem did not de-

Table 1. Hemodynamic changes.

Time (min)	5	10	15	20	25	30
Cardiac output (ml/min)						
Control	30.2±0.3	29.9±0.5	29.4±0.7	27.5±2.1	27.4±2.3	27.4±1.8
Diltiazem	29.5±0.2	26.4±2.0	24.5±3.2	21.5±4.1	21.1±4.1	20.5±4.0
Nicardipine	29.7±0.3	29.7±0.3	30.0±0.3	25.9±2.9	24.2±3.6	24.3±3.6
Verapamil	30.4±0.2	25.2±2.4	21.1±2.9+*	16.5±3.7+	17.0±3.8+	17.5±3.7+
Heart rate (beats/min)						
Control	280±7	288±6	287±8	289±11	280±8	284±13
Diltiazem	286±6	275±6	277±9	209±36	202±39	201±39
Nicardipine	279±7	285±5	291±7	229±31	224±35	228±35
Verapamil	284±10	273±7	261±8	203±32	213±33	207±32
Systolic Blood Pressure (mmHg)						
Control	103±2	97±1	92±3	92±5	95±4	98±3
Diltiazem	100±3	103±14	88±7	79±13	75±15	74±15
Nicardipine	99±2	94±1	93±2	86±9	80±12	78±12
Verapamil	110±2	92±6	81±6	66±12	65±11	68±12
Right atrial pressure (cmH_2O)						
Control	4.5±0.2	4.8±0.2	5.0±0.3	4.8±0.3	4.9±0.3	5.0±0.3
Diltiazem	3.9±0.2	4.5±0.3	4.6±0.4	4.6±0.5	4.1±0.3	4.2±0.3
Nicardipine	4.8±0.2	5.0±0.2	5.2±0.3	5.5±0.2	5.3±0.2	4.9±0.3
Verapamil	4.3±0.3	5.4±0.4	6.0±0.4	6.1±0.4	5.7±0.3	6.0±0.4
Left ventricluar dP/dt maximum (mmHg/sec)						
Control	5808±253	6337±362	6453±498	6350±496	6427±486	6518±422
Diltiazem	5885±400	5466±465	5524±638	4440±857	4250±934	4224±933*
Nicardipine	5078±385	5449±405	5472±439	4637±561	4182±752	4092±710*
Verapamil	4911±376	4439±569	4220±656	3162±706	3153±722	3263±724*

Time : after the start of perfusion.
The coronary artery was ligated at 8 min, and drugs were administered at 13 min.
* $P<0.05$ vs Control, + $P<0.05$ vs values at 5 min.

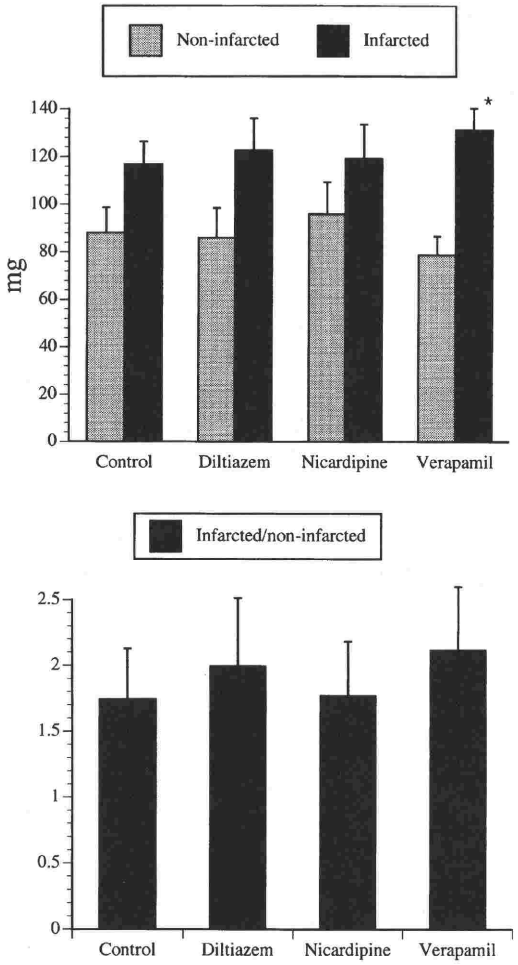

Fig. 1. Effects of Ca antagonists on the weight of myocardium and the ratio of infarcted and non-infarcted regions. The infarcted size was expressed by weight of the dissected myocardium from the infarcted region.
*P<0.05 vs. Non-infarcted region.

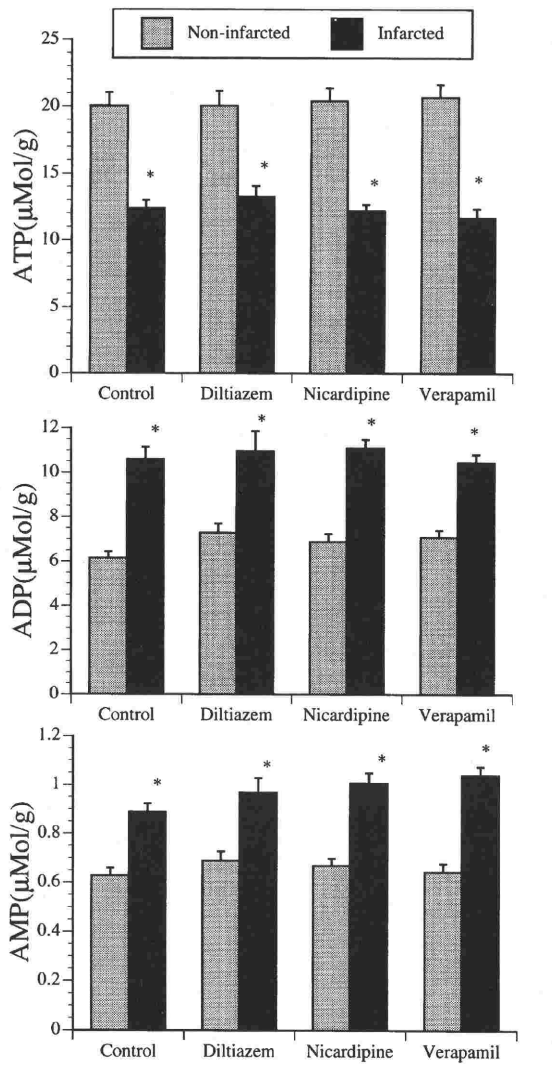

Fig. 2. Effects of Ca antagonists on high energy phosphates (ATP, ADP, AMP) in myocardium.
*P<0.05 vs. Non-infarcted region.

crease CO significantly in the same rat heart-lung model[13]. These drug concentrations are higher than those we employed in the present study. However, our results demonstrated that therapeutic doses of three calcium antagonists depressed LV dP/dt max in the infarcted rat heart. Furthermore, verapamil decreased CO after coronary ligation. These results are consistent with the review that most of the calcium antagonists available may adversely affect left ventricular function on infarcted heart[14]. In clinical studies, three large trials of calcium antagonists were undertaken in patients with late after myocardial infarction[15~17]. In two of these[16,17], mortality and the incidence of major coronary events after myocardial infarction were reduced. However, the benefit was particularly evident in those patients who were without heart failure. Recently, Psaty and colleagues[18] have reported that the use of short-acting calcium antagonists (nifedipine, diltiazem, verapamil) for patients with fatal or nonfatal myocardial infarction and hypertension might increase risk of myocardial infarction. Although extrapolation of our animal study to human clinical experience

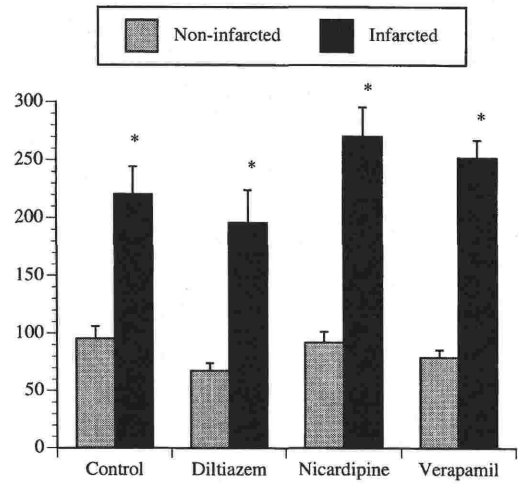

Fig. 3. Effects of Ca antagonists on myocardial lactate/-pyruvate ratio.
*P<0.05 vs. Non-infarcted region.

should be limited, it is likely that calcium antagonists may cause heart failure in the infarcted heart.

In spite of clinical disappointing results[4,5,14,18], some experimental evidence accumulated from animal models supports the ability of calcium antagonists to reduce both myocardial infarct size and the incidence of ventricular arrythmias[4]. In this study, there was no significant difference in the weights of infarcted regions produced by ligation among the groups. We visibly divided the myocardium into infarcted and non-infarcted regions at the end of perfusion. This method was not so accurate because we could not divided the inside of the myocardium perfectly. We should have injected the dye into the heart before dividing. Coronary perfusion in the V group might be worse than the other groups because verapamil tended to reduce CO and SBP more, although these were not statistically significant. This may be the reason why the weight of infarcted region in the V group was significantly heavier than that of non-infarcted region. We administered calcium antagonists 5 min after the coronary ligation. If we did before coronary ligation, there might be difference in the weights of infarcted regions among the groups because drugs had been reached to the infarcted regions.

All three calcium antagonists employed in this study have been reported to have beneficial effects on myocardial metabolism in ischemic models of isolated hearts[1~3,19]. These effects may result from a decrease in myocardial oxygen demand as well as an inhibition in transmembrane flux of calcium into the myocyte and preservation of sarcolemmal membrane permeability[20]. Previously, we have reported that nicardipine improved both functional and metabolic recovery from global ischemia in the same heart-lung preparation[3]. However, in this study, calcium antagonists did not influence myocardial metabolism during coronary ligation. This may be due to difference of types of ischemia, that is global or regional. In global ischemic model, given drugs were reached to whole heart after reperfusion, while in regional ischemic model drugs were not reached to the infarcted regions.

Although any extrapolation of these experimental data must be made with caution, results of the present study support the clinical experiences that most of calcium antagonists available are not clearly safe in patients with myocardial infarction.

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