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The Effect of Hyperkalemic Cardioplegia Containing Nitroglycerin or Diltiazem Hydrochloride on Hypertrophied Myocardial Protection.

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Abstract A hypertrophy heart may be more susceptible to ischemia during cardiac operations and require better myocardial protection than a normal heart. We hypothesized that cardioplegia containing vasodilator may be effective for hypertrophied myocardial protection. This study was designed to test this hypothesis. Ascending Aorta of Japanese white rabbits, weighing about 1kg, was banded (AoB) to induce myocardial hypertrophy. Three to four months later from AoB, the isolated heart was perfused using the Langendorff technique with Krebs-Henseleit bicarbonate buffer solution for 15 min, and then preserved for 2 hours in cardioplegic arrest by infusing an initial bolus of 20 ml of 4℃ crystalloid cardioplegic solution. The hearts were divided into three groups. Cardiac arrest was induced with modified Yamaguchi university crystalloid cardioplegic solution in Group A (n = 10), with that containing nitroglycerin (10^{-4} mol/L) in Group B (n = 6), and with that containing Ca^{2+} antagonist (diltiazem hydrochloride; 0.5mg/L) in Group C (n = 6). The pre and postischemic left ventricular developed pressure (LVDP), left ventricular end-diastolic pressure (LVEDP) and coronary flow were measured. Heart weight as well as the ratio of heart to body weight were significantly greater in AoB rabbits than those in the body weight matched rabbits ($p<0.05$). Percent recovery of LVDP was significantly higher in Group B after reperfusion than in Group A ($p<0.05$). Percent recovery of coronary flow was significantly greater in Group B than that in Group A at 15, 30 and 45 minutes after reperfusion ($p<0.05$). LVEDP was significantly lower in Group B after reperfusion than that in Group A ($p<0.01$). Percent recovery of coronary flow was significantly greater in Group C than that in Group A at 15, 30, 45 and 60 minutes after reperfusion ($p<0.05$). Hyperkalemic cardioplegia containing nitroglycerin was effective for protection of the hypertrophied myocardium.

Key words: Hypertrophied myocardium, Myocardial protection, Nitroglycerin, Diltiazem hydrochloride

Introduction

Hearts are hypertrophied with age as well as long term overload to the heart. Cardiac hypertrophy is characterized by increase in the heart to body weight ratio and by the dimensions of the myocytes making up the heart (1). The hypertrophied heart is more susceptible to ischemia during cardiac surgery, so that better myocardial protections

are requied. Because the consequential reduction of coronary reserve, i.e., reduction of blood flow per unit mass of tissue and increased wall mass, the metabolic requirements are increased in the hypertrophied myocardium (2). In addition, the thickness of the ventricular wall and the elevated left ventricular end-diastolic pressure (LVEDP) limit subendocardial coronary flow and contribute to inadequate distribution of cardioplegia(3). I hypothesized that cardioplegia containing a vasodilate drug might be effective for hypertrophied myocardial protection.

The present study was designed to determine the effect of nitroglycerin or diltiazem hydrochloride to a cold crystalloid cardioplesic solution, using a hypertrophied rabbit heart induced by banding of the thoracic aorta.

Materials and Methods

Cardiac hypertrophy model

Japanese white rabbits, weighing about 1 kg (1 month after birth), were anesthetized with 5 mg/kg of ketamine intramuscularly and 10 mg/kg of sodium pentobarbital, administered through the marginal ear vein. After orotracheal intubation, the animals were mechanically ventilated with 1-2% halothane in oxygen. After anesthesia, the ascending aorta was exposed by right thoracotomy, put on a cloth tape and banded by suturing the tape with 6-0 monofilament polypropylene (AoB). The banding was performed carefully to keep some amount of aortic blood flow, in other word the taping was not too tight. After AoB, a chest drain was inserted and was aspirated for approximately 1 hour. Thirteen to 16 weeks after AoB, the rabbits weighed 2.5-3 kg. Institutional guidelines for the care and use of laboratory animals were adhered throughout the experiment.

Experimental Protocol

Thirteen to 16 weeks after AoB, the rabbits were anesthetized in the same fashion as described above. The thorax was opened and 1000 IU of heparin was given intravenously. Thirty seconds later, the heart was excised and promptly placed in a cold Krebs-Henseleit bicarbonate buffer (KHBB, Table 1) bath. The aortic root was cannulated with a polyethylene cannula. A venting cannula was placed at the apex of the left ventricle (LV). A latex balloon mounted on a catheter was positioned in the LV through the mitral valve, to measure the left ventricular pressure under isovolumetric conditions. The isolated heart was suspended in a perfusion circuit and perfused through the aortic root at a pressure of 80 cmH_2O by the Langendorff technique using KHBB at 37℃, equilibrated with 95% O_2 and 5% CO_2. After instrumentation and stabilization for 15min, baseline measurements of isovolemic LV function and coronary flow were taken. The left ventricular pressure was measured by a Statham P23ID pressure transducer and recorded on a strip chart (Polygraph 362-2, Nihon Denki Sanei, Tokyo, Japan). The left ventricular developed pressure (LVDP), LVdP/dt and left ventricular end-diastolic pressure (LVEDP) were recorded as the balloon volume was increased in increments of 0.1-ml from 0 to 0.5 ml. Coronary flow was measured at the baseline balloon volume by the timed volumetric collection of effluent from the coronary sinus. After the baseline measurements were completed, the isolated heart was preserved for 2 hours in cardioplegic arrest by infusion of an initial bolus of 20ml of 4℃ crystalloid cardioplegic solution through the aortic root at a pressure of 80 cmH_2O followed by administration of 10ml/kg infusions of cardioplegic solution every 20 min to maintain cardioplegic arrest. The hearts were divided into three groups. In Group A (n = 10), cardiac arrest was induced and maintained with modified Yamaguchi university crystalloid cardioplegic solution (Table 2), in Group

Tabie 1. Composition of Krebs-Henseieit bicarbonate buffer

NaCl	118 mM
KCl	4.7 mM
$CaCl_2$	2.55 mM
$MgSO_4$	1.18 mM
KH_2PO_4	1.18 mM
$NaHCO_3$	24.88 g/L
Glu	11.1(37℃)

B (n = 6) with the crystalloid cardioplegic solution containing vasodilator; NTG (10^{-4} mol/L), and in Group C (n = 6) with the crystalloid cardioplegic solution containing calcium antagonist ; diltiazem hydrochloride (0.5mg/L). During the arrest, the myocardial temperature was maintained at 10℃ in cardioplegic bath. After 2 hours of the arrest, the heart was reperfused with KHBB at 37℃ in the perfusion circuit for 60 minutes. The postischemic LVDP, LVEDP, and heart rate were measured at 30 minutes after reperfusion under the same conditions as the baseline measurements. Coronary flow was measured at 15, 30, 45 and 60 min after reperfusion at a perfusion pressure of 80cmH_2O. The results of LV function and coronary flow were expressed as percent recovery that was calculated by the following formula:

percent recovery (%) = level after reperfusion/level at baseline×100.

The concentration of creatinine phosphokinase and its isoenzyme (CK, CK-MB), lactate dehydrogenase (LDH), and glutamic oxaloacetic transaminase (GOT) in the coronary effluent were measured at 30 minutes after reperfusion by the Ultra-Violet method.

Table 2. Composition of modified Yamaguchi universty crystalloid cardioplegic solution.

Na^+	85.3	mEq/L
K^+	25	mEq/L
Cl^-	85.5	mEq/L
Ca^{++}	0.5	mEq/L
Mg^{++}	10	mEq/L
Glu	25	g/L
PH	7.38	(37℃)
Osm	360	mOsm/L

Statistical Analysis

Statistical analysis was performed using two-way analysis of variance (ANOVA) for comparisons between groups and then Student's unpaired t-test at each balloon volume, using post-reperfusion values. All values are expressed as the mean ± standard error with a difference between two values being considered significant if the p value was less than 0.05.

Results

Degree of hypertrophy

There was no significant differences in body weight between normal heart rabbits and AoB rabbits. Heart weight as well as the ratio of heart to body weight were significantly greater in AoB rabbits than those in rabbits matched by body weight (Table 3).

Coronary flow

Percent recovery of coronary flow was significantly greater in Group B than that in Group A at 15, 30 and 45 minutes after reperfusion. It was significantly greater in Group C than that in Group A at 15, 30, 45 and 60 minutes after reperfusion (Fig.1).

Isovolemic LV function

There was no significant differences in baseline LV function among three groups (Table4).

Percent recovery of LVDP after reperfusion was significantly higher in Group B than that in Group A at balloon volum of 0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml, and 0.5 ml ($p<0.05$) (Fig.2). However, there was no significant differences in percent recovery of LVDP between Group A and Group C (Fig.2). There

Table 3. Weights of heart and body, and the ratio in rabbits

	Heart weight (g)		Body weight (g)		Heart weight/Body weight ratio (%)
H	9.029±0.526	*	3130.0±169.3	NS	0.295±0.007 *
H	12.544±1.271		2974.4±204.3		0.411±1.270

N: normal heart
H: hypertrophied heart mean±SE * $p<0.05$

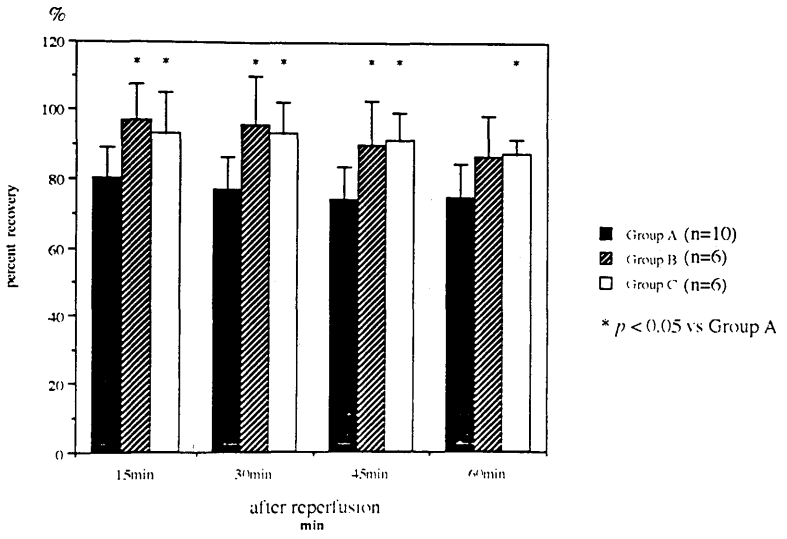


Fig.1 Percent recovery of coronary flow.
Group B and Group C demonstrated better recovery in coronary flow than Group A.

Table 4 Baseline measurements of coronary flow and left ventricular functions in hypertrophied rabbit hearts

	Group A	Group B	Group C
	coronary flow		
(ml/min)	47.9±7.2	52.0±11.9	46±2.7
	LVDP; mmHg		
ballon volume (ml)	control	NTG	Diltazem
0	63.7±7.8	51.3±16.5	60±4.9
0.1	63.1±7.6	55.0±14.5	62.7±10.5
0.2	68.8±9.4	63.7±9.5	69±3.4
0.3	71.9±10.0	65.4±10.4	70±1.2
0.4	71.1±9.6	70.8±5.1	69.5±13.4
0.5	68.2±7.9	74.7±4.6	72.2±12.8
	LVEDP; mmHg		
0.1	2.7±1.1	2.8±2.7	3.2±2.5
0.2	6.3±2.1	5.5±10.4	7.3±4.1
0.3	9.8±2.8	8.3±3.6	10.2±3.9
0.4	15.2±3.6	12.1±3.9	13.4±4.9
0.5	21.7±2.3	19.3±2.8	20.5±7.6
	LVdP/dt; mmHg/s		
0	950±70	1000±120	900±150
0.1	1150±60	1050±110	1100±100
0.2	1000±50	1000±150	890±250
0.3	1200±100	1150±100	960±150
0.4	1250±70	1200±130	1000±70
0.5	900±100	850±150	900±250

LVDP; left ventricular developed pressure,
LVEDP; left ventricular end diastolic pressure. mean±SE

was no significant differences in dP/dt among three groups at 30 minutes after reperfusion (Fig.3). LVEDP elevated significantly after reperfusion at balloon volum of 0.2 ml and 0.3 ml ($p<0.05$), and 0.4 ml and 0.5 ml ($p<0.01$) in Group A (Fig.4-1). However in Group B, LVEDP did not elevate after reperfusion(Fig.4-2). In Group C, significant elevation of LVEDP was demonstrated at balloon volume of 0.3, 0.4, and 0.5 ml ($p<0.05$) (Fig.4-3). There was significant differences in post reperfusion LV function but not in coronary flow between Group B and Group C.

Total enzyme release

There was no significant differences in the concentration of CPK, CPK-MB, LDH and GOT in the coronary effluent among three groups (Fig.5).

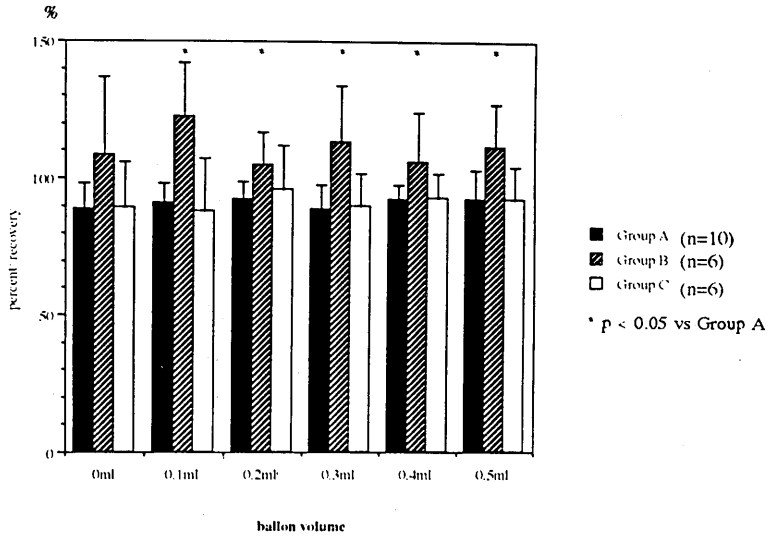


Fig. 2 Percent recovery of left ventricular developed pressure.
Group B demostrated better recovery in left ventricular developed pressure.

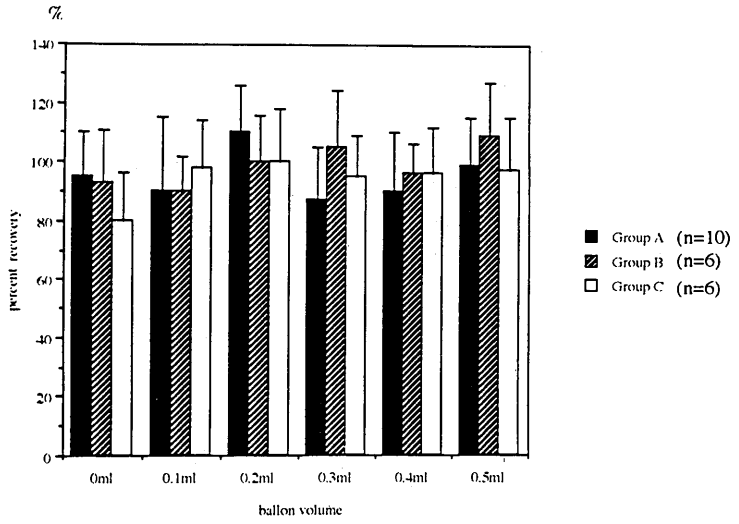
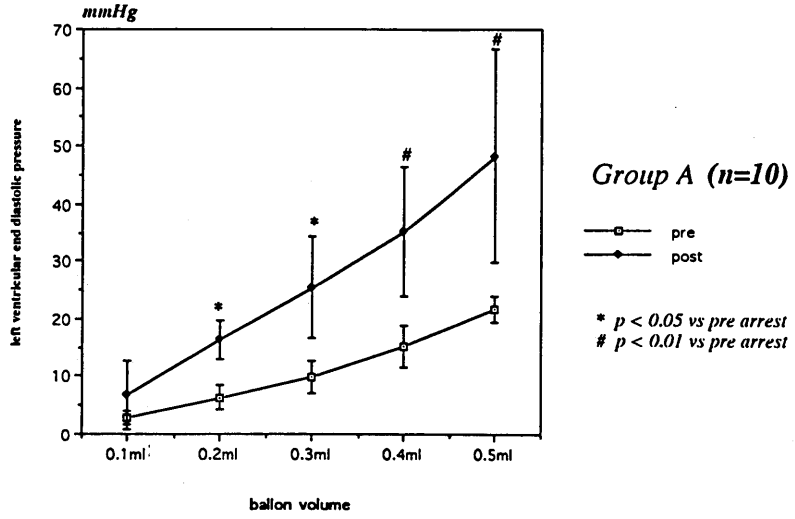


Fig.3 Percent recovery of left ventricular dP/dt
There was no significant differences between three groups after reperfusion.



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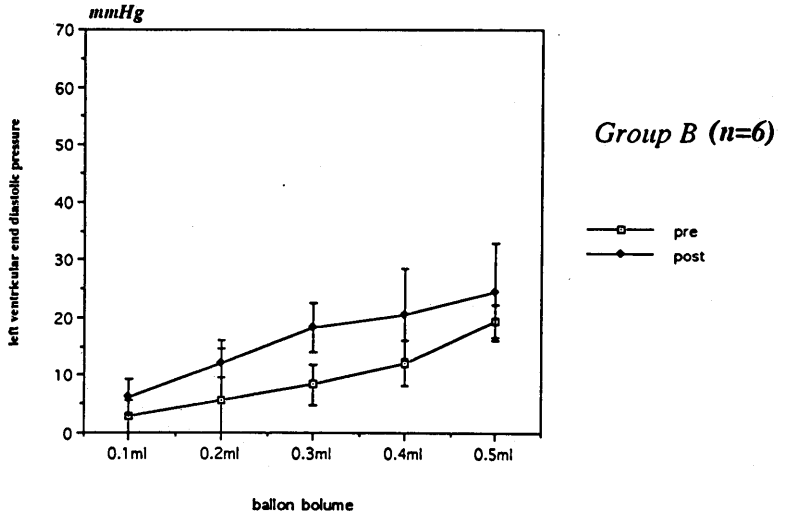

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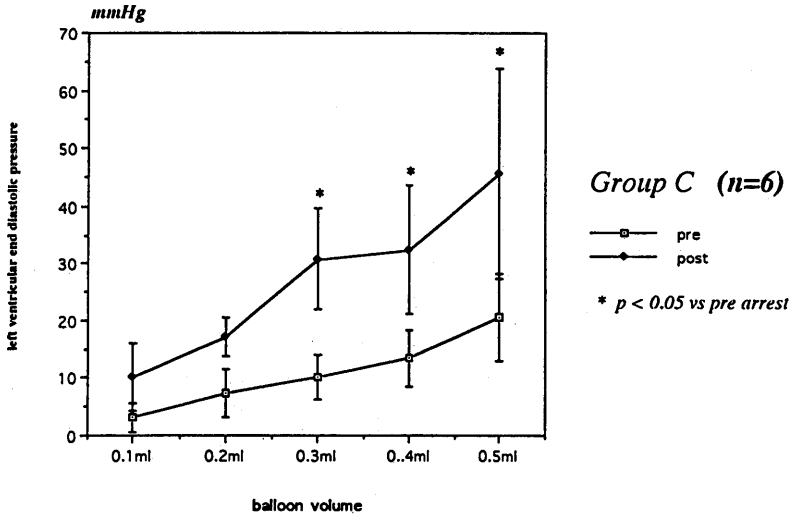

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Fig.4 Left ventricular end diastolic pressure.
Group B demonstrated better recovery in left ventricular end diastolic pressure than Group A and Group C.

Discussion

The hypertrophied myocardium induced by AoB, resulted in narrowing intra-LV capacity, namely "concentric hypertropy" (4). This occurs in a pattern that partially offsets the increase in ventricular wall stress. However, the cross sectional area of the capillary bed does not increase in parallel. This may lead to relative vascular insufficiency which combines with perivascular fibrosis, or "chronic hibernating myocardium". There are several experimental models of hypertrophied myocardium, such as thoracic aortic constriction described by Ryder et al (5), goldblatt method by Keung et al (6), congenital persistent truncus arteriosus method by Creazzo et al (7), abdominal aortic constriciton method by Scamps et al (8), growth hormone secreting tumor cells method by Xu and Best et al (9) and spontaneously hypertensive rat; Wistar-Kyoto rat by Brooksby et al (10). Ryder et al (5) have shown that thoracic aortic constriction leads to severe hypertrophied myocardium. For this experimental study, we used a model of hypertrophied myocardium induced by banding the ascending aorta of rabbits. We found that heart weight as well as the ratio of heart to body weight was significantry greater in AoB rabbits than those in non treated rabbits (Table 3), indicating that the hearts were hypertrophied.

The hypertrophied myocardium may be more susceptible to ischemia even in the absence of coronary artery disease. Addition of a vasodilator to cardiplegic solution may result in better perfusion of cardioplegia to the hypertrophied myocardium including endocardium, which leads better myocardial protection. In the present study, we studied two different drugs added to cardioplegia, i.e., NTG that acts vascular smooth muscle and induces vasodilation, and diltiazem hydrochloride that inhibits calcium influx into myocytes as well as cells of coronary vasuculature and possibly inhibits vasoconstriction and myocardial contraction. The results of this study show the protective ability of hyperkalemic crystalloid cardioplegia containg NTG to the hypertrophied myocardium. NTG has been used for more than 200 years to control the anginal syndrome associated with coronary insufficiency. Although the action of NTG on the systemic circulation has been known, the effects of NTG on the coronary circulation are still poorly understood (11). The known effects of NTG on vascular smooth muscle (12) suggest that the use of intracoronary administration of NTG results in dilatation of coronary vascular bed, and effectively offset the natu-

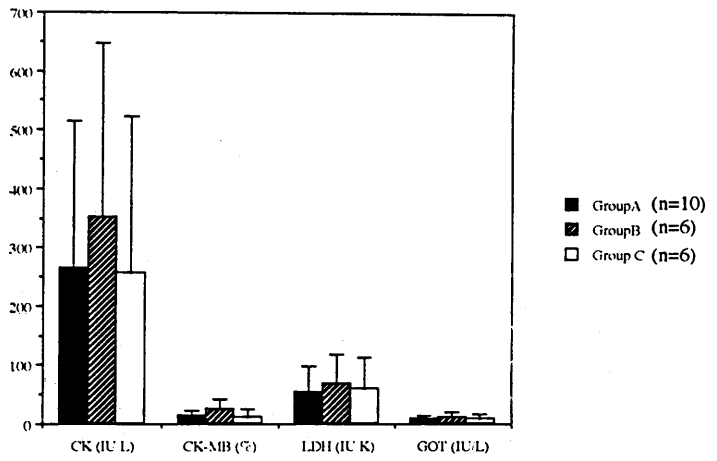

Fig.5 Creatinin phosphokinase(CK), CK-MB, lactate dehydrogenase (LDH) and glutamic oxaloacetic transaminase (GOT).
There were no significant differences in the levels of CK, CK-MB, LDH, and GOT among three groups after reperfusion.

ral rise in coronary vascular resistance associated with hypothermia. McKeon et al (13) demonstrated that hyperkalemic cardioplegia containing NTG at hypothermia has been decreased by 21.2% of coronary resistance in normal heart. In the present study, coronary flow demonstrated better recovery after reperfusion in Group B (coronary flow increased by approximately 20%; Fig 1) compared with Group A , suggesting that NTG reduced coronary resistance even in the hypertrophied heart. The maintenance of coronary flow may contribute to the better preservation of LV function (e.g, LVEDP, LVDP; Fig 2 and 4). Landymore et al (14) suggested that NTG cardioplegia may offer increased protection for the subendocardium during ischemia in normal human heart. Fam and McGregor (15) demonstrated that NTG increases the perfusion ratio of endocardium to epicardium and reverses subendocardial ischemia, which may contribute to the better recovery of left ventricular function in Group B compared with Group A. The higher rates of cardioplegic delivery achieved with NTG did not cause systemic hypotension during cardiopulmonary bypass despite the use of pharmacological doses. In addition, when NTG cardioplegia was used for the patients with coronary artery stenosis, coronary steal phenomena was not found from regional temperature data after infusion of cardioplegic solution (14). There was no significant difference in dP/dt between in Group A and Group B, but LVEDP was significantly lower in Group B than that in Group A (Fig 3 and 4). Therefore, we considered that expansibility was well preserved in Group B compared with that in Group A.

In the present study, coronary flow recovered well in Group C as in Group B, which might be because diltiazem hydrochloride prevented calcium influx to vascular smooth muscle cells, leading coronary vasodilation. Mori et al (16) demonstrated that Ca^{2+} antagonist, nicardipine, added to cardioplegic solution had myocardial protective effect in normal hearts. However, there was no significant difference in LV function between Group A and Group C (Fig 2, 3 and 4). The reason might be that diltiazem hydrochloride also prevented calcium influx to myocyte as well, resulting in a degree of contractility depression especially in hypertrophied myocardium. Sasaki et al (17) reported the results of clinical study that there was a little myocardial protective effect of diltiazem hydrochloride in cardioplegic solution in patients with hypertrophied hearts due to aortic stenosis.

In conclution, hyperkalemic cardioplegia containing nitroglycerin was effective for the protection of the hypertrophied myocardium.

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