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Experimental Study of Cold Blood Cardioplegic Solution. Is Cold Blood Cardioplegic Solution made Effective by the Red Blood Cell?

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Abstract This study was designed to distinguish whether the efficacy of blood cardioplegic solution is due to a cellular or plasma component. Japanese white rabbits' hearts were preserved for 3 hours by coronary perfusion with oxygenated crystalloid cardioplegic solution (group C), oxygenated blood cardioplegic solution (group B) and oxygenated crystalloid cardioplegic solution with plasma (group P). The coronary perfusion pressure was maintained at 20 cmH_2O, and myocardial temperature was maintained at 4℃. The isovolemic left ventricular functions after preservation in group B were better than those in group C. Coronary resistance after preservation in group C increased significantly compared to that in group B. O_2 consumption, perfusate CPK-MB levels, ultrastructural appearance and water content did not differ significantly among three groups. The blood cardioplegic solution was better for protecting the myocardium against ischemia. This efficacy might be not due to the oncotic pressure of plasma protein and oxygen supply but to some other properties of red blood cell, for example, as microparticles which facilitate capillary perfusion.

Key Words : Cold Blood Cardioplegia, Oncotic Pressure, Plasma, Red Blood Cell

Introduction

The efficacy of blood-based cardioplegic solutions in protecting the myocardium from ischemia has been generally accepted.[1] However, in hypothermia, the oxyhemoglobin dissociation curve shifts to the left[2] so that blood cardioplegic solution can not effectively deliver oxygen to the myocardium beyond that which is physically dissolved in the vehicle. On the other hand, blood cardioplegic solution contains plasma protein which exerts oncotic pressure, that drives fluid across the wall of the capillary. We hypothesized that the oncotic pressure of a blood cardioplegic solution minimizes myocardial edema, and that protection of the myocardium by blood cardioplegic solution is not due to the red blood cell but to the effect of oncotic pressure. The present study was performed to distinguish whether the efficacy of blood cardioplegic solution is due to a cellular or plasma component.

Materials and Methods

Japanese white rabbits, weighing 2kg, were used. Anesthesia was induced using intramuscular ketamine (15 mg/kg) and maintained with halothane after intubation. Ventilation

was controlled with a pressure-limited ventilator. After laparotomy, the inferior vena cava and the abdominal aorta were cannulated using a polyethylene cannula. After heparin^{R} (3 mg/kg) was injected intravenously, blood was withdrawn for use as blood cardioplegic solution. The heart was excised through a median sternotomy and placed promptly in a cold saline. The aortic root was cannulated with a polyethylene cannula, and then 20 mL of cold crystalloid cardioplegic solution was infused into the aortic cannula. A left ventricular venting tube was placed through an apical stab wound and used to divert Thebesian flow. A saline latex balloon mounted on a catheter was inserted into the left ventricle through the left atrium to measure left ventricular pressure (LVP) under isovolemic conditions.[3] The heart was mounted on a perfusion apparatus and then perfused via the aortic root at a pressure of 100 cmH_2O by the method of Langendorff with Krebs-Henseleit bicarbonate buffer (KHBB) [consisted of the following (in mM): NaCl 119.0, $NaHCO_3$ 25.0, KCl 4.6, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 1.3, and glucose 11.0.] at 37℃, equilibrated with 95% oxygen and 5% carbon dioxide. Coronary flow was measured by timed volumetric collection of effluent from the coronary sinus. Left ventricular pressure was measured using Uniflow Dispo Transduser^{R} (Baxter) and recorded on a strip chart (Polygraph 362-2, Nihon Denki Sanei Inc., Tokyo)(Fig. 1). After instrumentation and 15 minutes' stabilization, a baseline measurement was taken. Left ventricular pressure and its first derivative (LVdP/dt) were recorded as the balloon volume was iucreased in 0.2 mL increments to 0.8 mL by adjusting its volume to give an end-diastolic pressure of 0mmHg. Preischemic control values of developed left ventricular pressure, LVdP/dt, LV end-diastolic pressure and heart rate were determined. Coronary flow was measured at the base balloon volume. Each heart was preserved for 3 hours by continuous coronary perfusion with oxygenated crystalloid cardioplegic solution (group C; n=10), oxygenated blood cardioplegic solution (group B; n=10) or oxygenated plasma cardioplegic solution (group P; n=10). The composition of each of the three perfusates is shown in Table 1. Then potassium chloride was added to achieve a concentration of 20 mEq/L of potassium, and $NaHCO_3$ was added to give a pH of perfusate at 7.4. Coronary perfusion pressure was kept at 20cmH_2O, the myocardial temperature was maintained at 4℃, and the perfusate pH was kept at 7.4. The volume of the perfusate used was 100mL, and was recirculated. When the perfusate pH decreased, it was regulated with bicarbonate. After 3-hour preservation, the heart was removed from the preservation apparatus and reperfused with KH-buffer (37℃) on the perfusion circuit for 30 minutes.

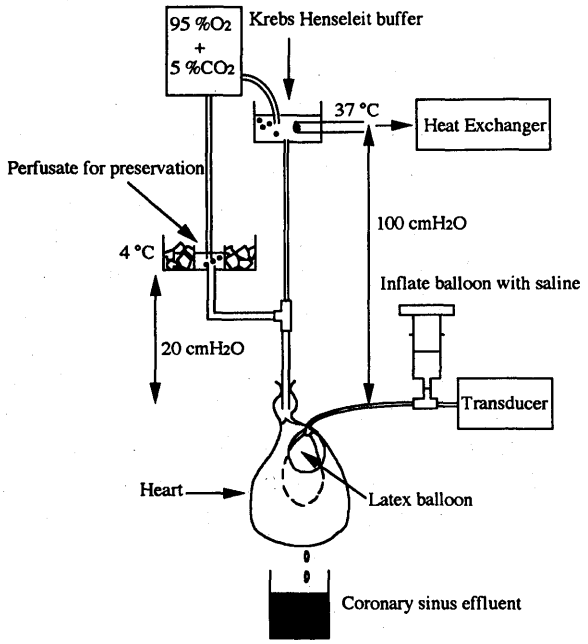

Fig. 1 Perfusion circuit.

Table 1. Composition of perfusate

Variable	Group C	Group B	Group P
Na(mM)	104	106	104
K(mM)	20.0	20.0	20.0
Cl(mM)	101	98	92
Ca(mM)	0.4	unknown	unknown
Mg(mM)	8.6	unknown	unknown
Glucose(g/L)	20.3	13.3	14.0
pH	7.46	7.38	7.43
Hb(g/dL)		4.2	
Osmolarity (mosm/L)	378	332	328
Oncotic pressure (mmHg)		8.3	8.3

Post-preservation left ventricular function was measured after 30 minutes' reperfusion under the same conditions as those under which the baseline measurement was made. A biopsy sample was taken from the left ventricular free wall after reperfusion to measure myocardial water content and evaluate any ultrastructural change. The total creatine phosphokinase isozyme (CPK-MB) level in the perfusate after preservation was measured.

Oncotic Pressure

A concentration of albumin and globulin in purfusates of group B and group P was measured. Oncotic pressure was calculated using the following equation:
Oncotic Pressure (mmHg) = 5.54×albumin+ 1.43×globulin[4]

Coronary Resistance

Perfusion pressure was maintained at 100cmH_2O. Coronnary resistance was calculated using the following equation: Coronary resistance (cmH_2O/mL/min.) =100/coronary flow

Oxygen consumption

Perfusate samples were withdrawn from the arterial and coronary sinus sources after 3 hours of preservation. Analyses of Perfusate's PO_2, PCO_2, Saturation O_2 and oxygen content were also made. Oxygen content and O_2 consumption were calculated by the following equation;

O_2 content (mL/dL.) =1.39×Hb(g/dL)× Sat(%)/100+0.003×PO_2(mmHg)[5),6)]

O_2 consumption (mL/min.) =[O_2 content (affluent) -O_2 content (effluent)]×coronary flow (mL/min.)/100

O_2 content (affluent); O_2 content of perfuusate to coronary artery

O_2 content (effluent); O_2 content of coronary sinus fluid

Myocardial Water Content

Samples were desiccated for 48 hours at 60℃. Then, water content was calculated using the following equation: myocardial water content (%) =(1-[dry weight/wet weight])×100

CPK-MB Release

The amount of CPK-MB released into the perfusate during a 3-hour preservation (per 1g of wet heart weight) was measured to determine the extent of myocardial injury. CPK-MB was measured by the UV method.

Ultrastrctural Appearance

A comparison of the ultrashuctural change in the heart in each of the three groups was conducted by evaluating the mitochondria. Mitochondrial scores were calculated by the method of Hashiba.[8)] For each heart, five electron micrographs of the endocardial layer were taken. In each micrograph, five mitochondrial patterns were graded with a score of 0 through 4, as described by each heart, five electron micrographs of the endocardial layer were taken. In each micrograph, five mitochondrial patterns were graded with a score of 0 through 4, as described by Flameng[9)] (Score 4, normal structure with well preserved mitochondrial granules, Score 3, normal structure but granules absent, Score 2, swollen mitochondria with clarification of the matrix, Score 1, disruption of mitochondrial crests with clarification as well as condensation of the matrix and Score 0, disruption of the crests and loss of integrity of mitochondrial inner and outer membranes.). Therefore, a total of 25 mitochondria were evaluated, and the mitochondrial score ranged from 0 through 100.

Data Analysis

Statistical analysis was performed using ANOVA, Scheffe F-test and Student's t-test. The values are expressed as the mean ±the standard error of the mean. Differences were considered significant when the P value was less than 0.05.

Results

Isovolemic Left Ventricular Function

No significant difference was found among three groups in baseline ventricular function (Table 2). The percent recovery of left ventricular developed pressure in group B was better at balloon volumes of 0.2 to 0.8 mL than in any other groups. At a balloon volume of 0.6 mL, the percent rccovery was 70.1±5.2% in group C, 95.4±6.8% in group B and 77.7±8.9% in group P. In group B, recovery tended to be better than that in group C ($p<0.1$, Table 3). The percent recovery of dP/dt after preservation in group B at a balloon volume of 0 to 0.8 mL, except 0.2 mL, was greater than that in any other groups, though there was no significant difference among three groups (Table 3). In particular, the percent recovery of -dP/dt after preservation at a balloon volume of 0.6 mL was 69.0±6.2% in group C, 102.1±10.6% in group B and 76.4±10.9% in group P. In group B at the balloon volume of 0.6 mL it was significantly better than that in group C ($p<0.05$, Fig. 2). Post preservation left ventricular end-diastolic pressure in group C increased significantly compared to the baseline value (pre-preservation value) at balloon volumes of 0.4, 0.6 and 0.8 mL ($p<0.05$). In addition, LVEDP in group P increased significantly compared to the baseline value at balloon volumes of 0.2 to 0.8 mL ($p<0.05$). On the other hand LVEDP in the group B did not increase significantly at 0.4 to 0.6 mL balloon volumes (Fig. 3). These findings indicate that the isovolemic left ventricular function in group B was preserved better than that of group C.

Oncotic Pressure

Oncotic pressure was 8.3±0.2 in group B(n=3), and 8.3±0.5 in group P(n=3).

Coronary Resistance

The values for coronary resistance (cmH_2O/mL/min) at baseline and at 30 minutes' reperfusion after preservation were, respectively 3.06±0.33, 5.03±0.61 in group C, 2.42±0.12 and 3.29±0.22 in group B and 2.77±0.32, 4.39±0.40 in group P. The post-preservation coronary resistance was significantly higher than the baseline value in any group ($p<0.05$). However, that in group C was higher than that in group B (Fig. 4). This indicates

Table 2. Left Ventricular Function at Baseline (Before Preservation). Date are the standard error of the mean. No significant differences were found in baseline measurements of left ventricular function among three groups.

Group	Balloon Volume (mL)	Variable (mean±SEM)				
		HR (beats/min)	Developed pressure (mmHg)	dP/dt (mmHg/s)	-dP/dt (mmHg/s)	LVEDP (mmHg)
Group C	0	142±17	39±7	626±94	-459±64	0
	0.2	142±17	54±8	785±105	-582±78	5±2
	0.4	146±15	61±8	810±108	-596±77	8±2
	0.6	141±16	63±7	963±108	-608±71	14±3
	0.8	141±16	64±7	845±117	-628±74	21±4
Group B	0	144±17	31±5	572±81	-455±90	0
	0.2	146±13	45±5	813±130	-538±91	7±2
	0.4	146±13	50±4	850±134	-562±78	15±4
	0.6	146±13	51±4	890±129	-563±77	23±6
	0.8	145±12	56±4	966±122	-598±80	31±8
Group P	0	165±10	41±7	814±137	-630±119	0
	0.2	165±10	56±9	1019±197	-800±158	6±2
	0.4	163±9	64±9	1089±217	-820±162	11±4
	0.6	161±9	66±9	1125±238	-803±155	15±4
	0.8	161±9	68±9	1132±228	-816±152	22±6

Table 3. Left Ventricular Function after Preservation. Date are shown as the mean ± the standard error of the mean. %Developed pressure in Group B tended to recover better than did in balloon volumes of 0.6 mL ($p<0.1$). %-dP/dt in Group B demonstrated better recovery at balloon volumes of 0.6 mL ($p<0.0,5$). HR; heart rate. % Developed pressure; Percent recovery of developed pressure. %dP/dt; Percent recovery of the maximum rate of rise of left ventricular pressure. %-dP/dt; Percent recovery of minimum rate of rise of left ventricular pressure.

Group	Balloon Volume (mL)	Variable (mean±SEM)				
		HR (beats/min)	%Developed pressure (%)	%dP/dt (%)	%-dP/dt (%)	LVEDP (mmHg)
Group C	0	143±12	110.7±34.3	104.6±31.2	92.5±32.2	0
	0.2	143±11	87.5±17.8	100.1±18.1	86.8±19.2	8±1
	0.4	142±12	76.6±9.4	101.4±15.3	90.3±19.0	15±2
	0.6	141±13	70.1±5.2	78.7±8.9	69.0±6.2	25±4
	0.8	141±13	68.0±4.6	78.2±7.7	70.1±7.3	33±4
Group B	0	146±13	96.6±13.2	117.8±25.2	105.4±18.9	0
	0.2	146±13	88.6±7.7	98.7±14.1	98.9±14.6	10±2
	0.4	145±13	90.7±9.9	110.3±19.7	102.5±11.8	17±3
	0.6	145±13	95.4±6.8	106.4±15.3	102.1±10.6	28±6
	0.8	143±13	90.4±6.8	83.5±5.2	91.6±8.6	43±9
Group P	0	143±13	86.9±20.9	83.4±16.1	80.7±21.4	0
	0.2	143±13	87.4±17.0	92.2±14.3	78.8±14.4	11±3
	0.4	143±13	87.4±13.7	86.8±12.3	77.2±11.1	17±5
	0.6	139±14	77.7±8.9	87.7±14.2	76.4±10.9	31±7
	0.8	140±14	74.4±9.9	80.9±12.1	71.3±10.0	41±8

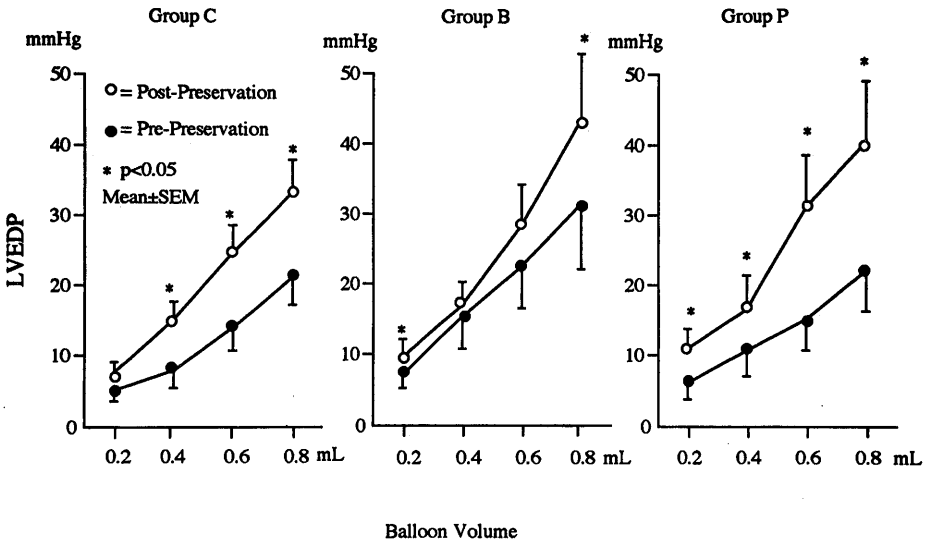

Fig. 2 Percent recovery of minimum rate of rise of left ventricular pressure. Group B demonstrated better recovery at balloon volumes of 0.6 mL ($p<0.05$). (SEM=standard error of the mean.)

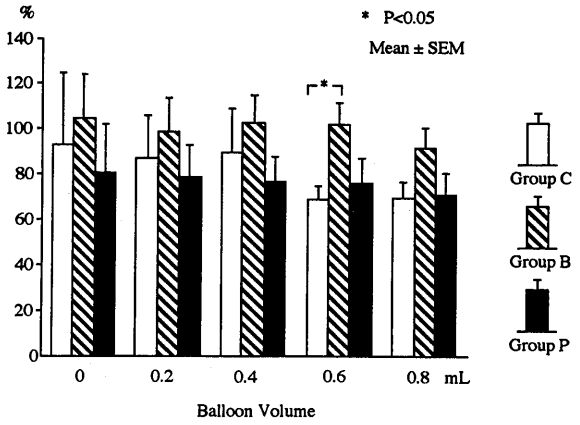

Fig. 3 Relationship between left ventricular end-diastolic pressure (LVEDP) and balloon volume. The LVEDP in group C increased significantly at balloon volumes of 0.4, 0.6 and 0.8 mL compared to the baseline value ($p<0.05$). The LVEDP in group P increased significantly at balloon volumes of 0.2, 0.4, 0.6 and 0.8 mL compared to the baseline value ($p<0.05$). On the other hand the LVEDP in group B increased at balloon volumes of 0.2 and 0.8 mL compered to the baseline value ($p<0.05$), with no significant difference at balloon volumes of 0.4 and 0.6 mL. (SEM=standard error of the mean.).

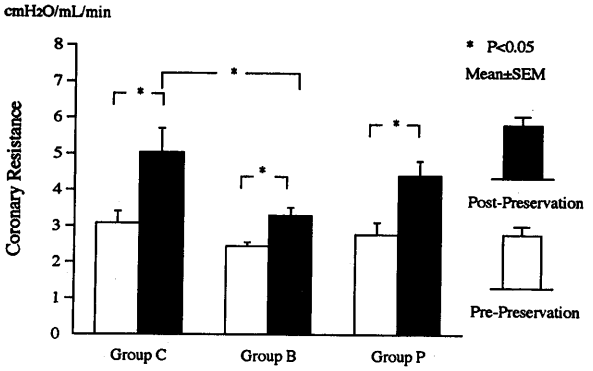

Fig. 4 Coronary resistance. There was a significant increase in coronary resistance after preservation among three groups compered to the baseline, with the group C coronary resistance being higher than in group B ($p<0.05$). (SEM=standard error of the mean.).

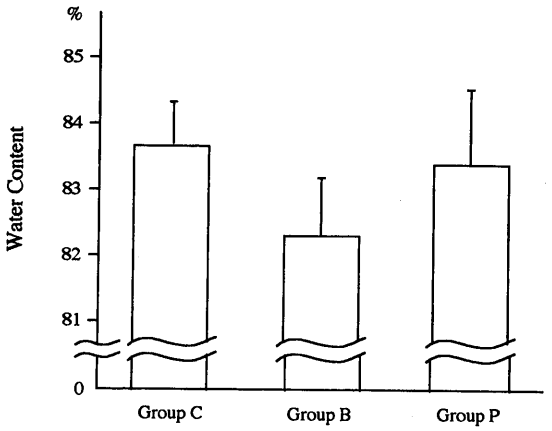

Fig. 5 Myocardial water content. There was no significant difference in myocardial water content among three groups. Values are shown as the mean ± the standard error of the mean.

that compliance of the left ventricle after preservation in group B was maintained better than it was in group C.

Myocardial Water Content

Myocardial water content after reperfusion was 83.7±0.58% in group C, 82.3±0.83% in group B and 83.4±1.07% in group P (Fig. 5). In group B, water content was lower than in any other group, however, there was no significant difference in myocardial water content among three groups.

Total Creatine Phosphokinase Release

The total amount of CPK-MB (IU) released into the perfusate was 1.38±0.61(IU/g) of heart in group C, 1.70±0.3OIU/g of left ventricle in group B and 2.00±0.58IU/g of left ventricle in group P (Fig. 6). There was no significant difierence in CPK-MB among the groups.

O_2 Consumption

O_2 consumption (mL/min./g) after 3 hours preservation was 1.67±0.42 in group C, 0.84±0.31 in group B and 0.75±0.18 in group P (Fig. 7). O_2 consumption in group C was greater than any other group, with no significant

differeuce in O_2 consumption among the groups.

Ultrastructural Appearance

In every group, there was moderate ischemic injury which was manifestated as slight mitochondrial swelling and cristal disruption. In groups C and P the cristal disruption was severe. In group B, however, the cristal disruption was moderate (Fig. 8). Mitochondrial scores aere 57.2±5.3 in group C, 65.0±4.7 in group B and 56.1±3.2 in group P. The mitochondrial score in group B was better than in any other group. However, there was no significant difference in mitochondrial score among three groups (Table 4).

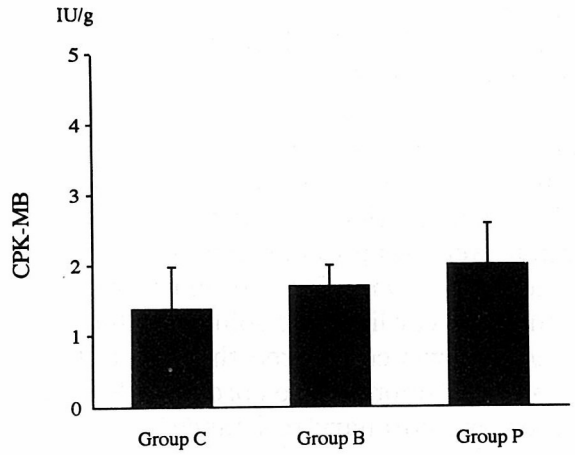

Fig. 6 CPK-MB release. There was no significant difference in CPK-MB release among three groups. Values are shown as the mean ± the standard error of the mean.

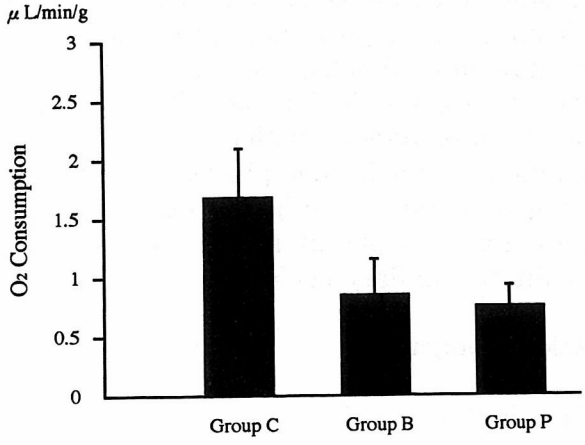

Fig. 7 Oxygen consumption. There was no significant differeuce in oxygen consumption among three groups. Values are shown as the mean ± the standard error of the mean.

Discussion

O_2 Consumption

Blood cardioplegic solution is generally accepted as effective in protecting the myocardium. Buckberg[1] showed that blood cardioplegic solution was effective with respect to the delivery of oxygen and maintenance of oncotic pressure. In hypothermia, however, the oxyhemoglobin dissociation curve shifts to the left[2] so that oxygen is made less readily available and requires

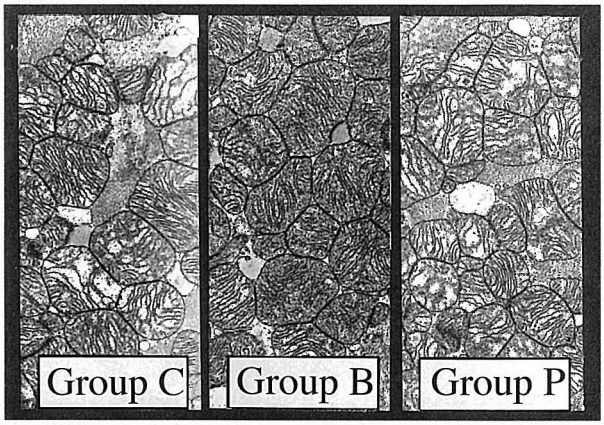

Fig. 8 Ultrastructural appearance. The hearts in every group sustained moderately ischemic injury, maintained as slight mitochondrial swelling and crystal disruption. In groups C and P the cristal disruption was severe, but was moderate in group B(Uranyl acetate and lead citrate; ×6,000)

Table 4. Mitochondrial score (mean ±SEM). There was no significant difference in myocardial water content among three groups. (SEM=standard error of the mean.).

group C	group B	group P
57.2±5.3	65.0±4.7	56.1±3.2

increasingly reduced tissue oxygen tension before oxygen is released. Therefore, blood cardioplegic solution does not effectively deliver oxygen to the myocardium beyond that which is physically dissolved in the vehicle. Therefore, the adverse effects of hypothermia, such as sludging and increased viscosity[10], may impair subsequent capillary distribution. On the other hand, hypothermia increases the affinity for oxygen and lowers energy requirements. The importance of myocardial temperature was emphasized in the work of Magovern and associates[11] who found that blood cardioplegic solution was most effective at 20℃, had no additional benefit (over crystalloid cardioplegia) at 10℃, and was inferior to crystalloid cardioplegic solution at 4℃. In the present study, our data showed same results. In the present study, O_2 consumption in group B was almost equal to that in group P. Thus, at hypothermia, hemoglobin could not make myocardial oxygen consumption increased. On the other hand, with respect to other parameters (isovolemic LV function, coronary resistance and water content), group B was better than any other group in spite of low O_2 consumption. So, the high O_2 consumption at preservation is not seemed to indicate bad myocardial protection.

Oncotic pressure

Blood cardioplegic solution contains plasma protein to provide oncotic pressure, which prevents the drive of fluid across the wall of the capillary. Thus, the oncotic pressure of blood cardioplegic solution is seemed to minimize myocardial edema and water content. In the present study, the oncotic pressure in group B was equal to that in group P. The water content in group B was less than that in group P. The water content in group C was not different from that in group P. Thus oncotic pressure does not minimize water content and affect greatly to myocardial protection.

The efficacy of blood cardioplegic solution may be attributed neither to greater oxygen delivery by the red cell nor to the oncotic pressure of plasma protein, but is likely to be related to some other red cell properties. There are several interesting reports regarding the capillary flow distribution of blood cardioplegic solution. Zweifach[12] reported that, when the circulating medium was crystalloid or colloid, capillary flow was not obtained. However, addition of red blood cells or carbon particles to the circulating medium resulted in complete perfusion of the capillary bed. Berkowitz[13] reported that glomerular filtration improved when nitrite -treated red cells (no oxygen transport function) were added to an oxygenated perfusion system. Blood cardioplegic solution has been reported by Robertson[14] to improve perfusion distal to a coronary stenosis than possible with crystalloid cardioplegic solution. These reports suggest that red cells, or other microparticles, facilitate capillary perfusion. According to our experimental observations of decreasing coronary resistance with blood cardioplegic solution after preservation, it was considered that the red biood cells led to improve the coronary flow distribution and coronary resistance.

Conclusion

We conclude that during 3 hours' preservation of isolated rabbit heart by continuous hypothermia and low pressure coronary perfusion, oxygen delivery by blood cardioplegic solution dose not exceed that possible with crystalloid cardioplegic solution, but is more effective at myocardial protection than crystalloid cardioplegic solution. The major factors responsible for the efficacy of the preservation possible with blood cardioplegic solution do not include plasma proteins and oxygen supply but other properties of red cells, for example, as microparticles which facilitate capillary perfusion.

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