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Intermittent Anterograde Normothermic Blood Cardioplegia: Experimental Study in Rabbits

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ABSTRACT

Background : We investigated the degree of myocardial protection provided by intermittent anterograde normothermic blood cardioplegia infusion for 60 minutes at 37° C in normal rabbit hearts.

Methods : Thirty-two New Zealand rabbits were studied and divided into two groups: experimental group and control group. In the experimental group, normothermic blood cardioplegia was infused into the aortic root every 20 minutes over a one-hour period using a two-minute infusion dose. This amounted to an ischemic (unperfused) time of 52 minutes (or 86.6% of the total time). The biochemical investigation was carried out in two phases; Phase I:metabolic study after ischemia with no reperfusion and Phase II: metabolic and functional study after reperfusion. Reperfusion was carried out using a parabiotic perfusion system. Myocardial glycogen and mitochondrial respiration in the ventricular myocardium were established immediately after the end of intermittent cardioplegic solution infusion (Phase I) and after blood reperfusion (Phase II), when left ventricular function (dP/dt_{max}) was also evaluated.

Results : At the end of Phase I, there was a significant decrease in myocardial glycogen levels to 58% compared with the control group. In Phase II, the differences in myocardial glycogen between the experimental and the control group were not significant. Mitochondrial respiration analysis did not show significant differences between the experimental and control groups, either in Phase I or II. In Phase I, dP/dt_{max} values were 903.39 ± 113.46 mmHg/sec and 1,043 ± 256.94 mmHg/sec for the experimental and control group, respectively. These differences were not statistically significant.

Conclusions :Intermittent anterograde blood cardioplegia infusion every 20 minutes for 60 minutes at 37° C was an effective myocardial protection method in normal rabbit hearts.

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INTRODUCTION

Hypothermia, first introduced experimentally in cardiac surgery in 1954 by Bigelow et. al., with later contributions by Shumway & Lower, has become an important and universally effective method of tissue preservation, both in extracorporeal circulation and myocardial protection [Bigelow 1954, Shumway 1959a, Shumway 1959b]. Others utilized hypothermia as an adjunct to the protective effects of cardioplegic arrest [Bernhard 1961, Buckberg, 1979, Buckberg 1987, Buckberg 1991]. Although hypothermia is still widely used in myocardial protection strategies, it is not free of hazardous effects during extended periods of ischemia. Several publications have reported the disadvantages of hypothermia, whether topical or in cardioplegic solutions, especially when trying to obtain myocardial temperatures below or near 10°C [Lazar 1980, Benjamin 1982, Kurihara 1983].

In 1991, Lichtenstein et. al. introduced the use of continuous normothermic blood cardioplegia and reported favorable clinical results [Lichentenstein 1991, Christakis 1992, Lichentenstein 1992]. However, during myocardial revascularization, continuous normothermic blood cardioplegia often must be interrupted in order to improve visualization of the target vessel. Several authors have recently presented excellent results with interrupted normothermic blood cardioplegia if the interruption times were not longer than 10 to 15 minutes [Pelletier 1994, Calafiore 1995, Mezzetti 1995].

The use of intermittent normothermic (37°C) blood cardioplegia has all the advantages of a continuous infusion with the potential additional benefits of better visualization, simplification and cost reduction. Another potential advantage of intermittent infusion is that it may avoid secondary fluid overload and hyperkalemia.

We believe that the infusion intervals may be safely extended based on the following facts:1) asystolic induction is aerobic, 2) the heart remains in asystole between infusion intervals, and 3) periodic reinfusion of oxygenated blood may restore energy reserves and eliminate the harmful metabolites which result from anaerobic metabolism.

Our experimental study was based upon the considerations above and was aimed at evaluating the degree of myocardial protection provided by intermittent hyperkalemic blood cardioplegia infusion every 20 minutes for 60 minutes at 37°C in a model of isolated rabbit heart perfusion.

MATERIALS AND METHODS

Animals

New Zealand male rabbits weighing 2 to 3 kg were used. The animals were divided into donors (n=32), receptors (n=9) and blood donors (n=17).

A donor was defined as the animal whose heart was taken for the experimental study. A receptor animal was defined as the rabbit connected to the parabiotic perfusion device. The blood donor is the animal whose blood was used to prepare cardioplegic solutions and for transfusions in the receptor rabbit.

Anesthesia

All of the animals were anesthetized with sodium pentobarbital IV at 30 mg/kg of body weight. The animals were intubated via cervical tracheostomy and controlled mechanical ventilation established using a pressure-cycled ventilator (Takaoka respirator, model 600) at 30 cycles/min with 100% oxygen.

Surgical Procedure

The donor cardiectomy was performed via a midline sternal and cervical incision. The thymus was resected and the aorta and its branches were dissected and all of them ligated with cotton threads. The ascending aorta was cannulated by one of the carotid arteries with a 16-ga catheter (Intracath) and the cardioplegic solution was infused after distal ligature of the aortic arch. Both atria were opened to avoid distension of the arrested heart. The cardiectomy was completed by transection of the vena cava, distal aorta, brachiocephalic vessels, pulmonary trunk, and pulmonary veins.

In the receptor rabbit, one carotid artery and one internal jugular vein were dissected free by cervical cutdown. After heparinization, both vessels were cannulated. A 16-ga Intracath was introduced into the carotid and threaded into the aorta. The jugular vein was cannulated with a polyethylene PE 240 tube. The arterial catheter was connected to a circulatory pump and the venous catheter to the venous drainage line, both part of the reperfusion device demonstrated in Figure 1 (◎).

For the blood donor rabbit, a midline laparotomy was created, the abdominal contents eviscerated and covered by a gauze dampened with 0.9% NaCl solution at 37°C. The infrarenal aorta was dissected and, after heparinization, was caudally sutured and cranially cannulated using a 16-ga Intracath. All the blood required for the cardioplegic solution was acutely and rapidly aspirated by this cannula. The remaining blood, about 50 ml, was removed for transfusion to the receptor animal. The acute exsanguination was aimed at avoiding hemodilution and acidosis through hypovolemia.

All of the animals received 3 mg/kg of heparin IV at the end of the surgical exposure and before vascular cannulation.

Cardioplegic Solution

The pharmacology of the cardioplegic solution is described in detail in Table 1 (◎).

Reperfusion System

The reperfusion system we utilized was originally suggested by Chen et. al. [Chen 1993] with some of our own changes as illustrated in Figure 1 (◎). The system was primed with 100 ml of Ringer's solution to which 15 mg of sodium heparin and 3 mEq of sodium bicarbonate were added. Before starting perfusion, 50 ml of arterial blood was transfused to the receptor to avoid hemodynamic disturbances. The perfusate temperature was maintained by water circulation at 37° C between the internal and external walls of the arterial reservoir and the collecting bottle.

During the ischemic period, the hearts received a cannula in the aorta and 2 epicardial pacing electrodes. A latex balloon catheter was introduced in the left ventricular cavity through the mitral valve. The mitral ring was sutured around the catheter to avoid extrusion during ventricular contractions.

Reperfusion was simultaneously carried out in two hearts. Five minutes after the spontaneous resumption of mechanical activity, electric stimulation was started at the rate of 180 beats/minute, with an amplitude of 20 mV, and only then the recording of intraventricular pressure was started.

At the end of the pre-established 20-minute reperfusion period, the ventricles were excised at the atrioventricular groove and immersed in cold 0.9% NaCl solution in Becker vials, placed in ice, and transported for processing and biochemical analysis.

Left Intraventricular Pressure Measurement and Analysis

Pressure curves were recorded using a 4-channel Physiograph MK-IV polygraph by Narco Bio Systems Inc. (Hous-

Table 1. Composition of cardioplegic solutions

	Induction	Maintenance
Ringer's Solution	12 ml	12 ml
Arterial Blood	8 ml	8 ml
(% of the total volume)	(40%)	(40%)
KCL	30 mEq/l	18mEq/l
Interval between	Only	20'/20'
Doses	(Time 0)	(20', 40', 60')
Dose volume	20 ml	20 ml

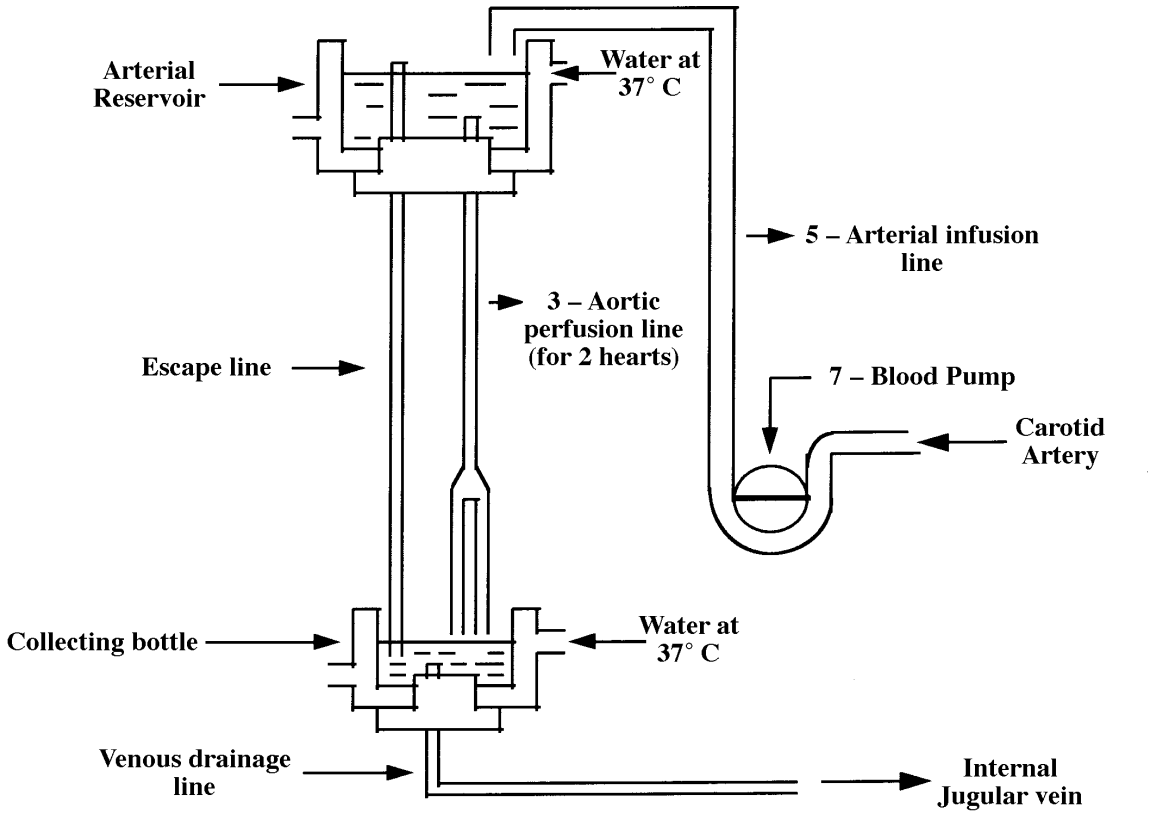

FIGURE 1. Parabiotic perfusion device.The blood aspirated from the carotid artery of the receptor rabbit is taken to the arterial reservoir and perfused retrograde into the aorta of the donor heart through the aortic perfusion line.The blood from the coronary sinus of the studied heart and the surplus of the pre-established level of the arterial reservoir are collected in the collecting bottle and re-infused to the receptor by the internal jugular vein.The perfusion pressure is constant and is established by the size of the perfusate column take from the arterial reservoir to the distal end of the aortic perfusion line.

ton, Texas, USA). Both hearts were recorded simultaneously in different channels, and had the same electric calibration and mercury manometer with a 0- to 200-mmHg scale (50 mmHg/cm). The pressure transducer was model 7179 with 8 microvolts/cm of sensitivity (Narco Bio Systems Inc., Houston, Texas, USA).

Left ventricular dP/dt values were calculated manually from intraventricular pressure tracings 10, 15, and 20 minutes after starting reperfusion according to the method published by Aloan [Aloan 1982]. At least three separate tracings were used to calculate a mean dP/dt value.

Metabolic Study

Glycogen levels: Glycogen extraction was carried out by the method suggested by Sjogreen et. al. using 500 mg of cardiac muscle obtained from a section of the apex of heart [Sjogreen 1938]. Dosage was carried out by the method suggested by Hassid & Abrahan [Hassid 1957].

Mitochondrial respiration: Respiratory Control Rate (RCR) was established and defined as the rate between oxygen consumption in Stage III (active phase of oxidative phosphorylation with oxygen consumption and ATP formation) and Stage IV (baseline respiration after ADP is phosphorylated into ATP). The ADP: O_2 ratio is given by the amount of oxygen required for total phosphorylation of a known ADP amount. This ratio gives an idea of phosphorylation and biological oxidation in the respiratory chain.

Mitochondrial fraction was obtained by Sordhal's technique [Sordhal 1971]. Mitochondrial oxygen consumption was carried out by polarography at 30° C, using a Gilson 5/6 Oxygraph (Gilson Medical Electronics, Inc., W. Beltline, Middeton, WI, USA) provided with a Clark oxygen electrode.

The mitochondria were shaken and placed in the chamber so as to obtain 1 mg of mitochondrial protein per milliliter of the respiration media. Mitochondrial protein was established by Lowry's method [Lowry 1951]. Alphaketoglutarate 5mM was used (Sigma Chemical Company, St. Louis, MO, USA), as the potassium salt. The stimulation for oxygen consumption (Respiration stage III) was induced by adding 400 nmoles of MgADP (Sigma Chemical Company, St. Louis, MO, USA).

Oxidative phosphorylation parameters were calculated according to Chance & Williams [Chance 1956] and Estabrook & Pullman [Estabrook 1967] and were expressed in oxygen nanoatoms per mg protein per minute for Stage III and IV of mitochondrial respiration.

Project Design

Phase I. Metabolic study after ischemia without reperfusion: In this phase, glycogen levels and mitochondrial respiration of 15 hearts undergoing intermittent blood cardioplegia at 37°C for 60 minutes were compared. Infusion time was about 2 minutes, with intervals of 20 minutes between each infusion. The hearts were divided into an experimental group (N=10) and and a control group (N=5).

Phase II. Metabolic and Functional study after r eperfusion: Seventeen hearts were studied in this phase. The experimental group (N=10), received the same treatment as the Phase I group until the end of the 60-minute period of intermittent cardioplegic infusion. During this period, the hearts were prepared for reperfusion. In the control group (N=7), reperfusion was carried out 5 to 7 minutes after the infusion of the first cardioplegia dose, this time interval was required for reperfusion preparation. In the meantime the hearts were maintained in 0.9% NaCl solution at 4°C.

Statistical Analysis

Student's T-test was used for result comparison, with a significance level of 0.01. Results were expressed as mean values ± SEM. Comparisons between the groups with and without reperfusion were not carried out since the groups are different, with different technical characteristics, such as the short ischemia period before reperfusion required to set the hearts in the perfusion device.

RESULTS

Myocardial Glycogen

Table 2 (◎) shows myocardial glycogen values for each group in Phases I and II and their respective control groups. Results at the end of the 60-minute period in phase I demonstrated a 58% decrease in myocardial glycogen levels compared to the control group. This difference was statistically significant. In Phase II, although glycogen levels were lower than the respective control group, this difference was not significant.

Mitochondrial Respiration

The results of the mitochondrial respiration parameters (Stage III and IV, RCR and ADP/O_2) are shown in Table 3 (◎) for Phases I and II. The differences were not significant.

Left Ventricular Function (dP/dt $_{max}$)

The dP/dt_{max} values measured during Phase II were 903.39 ± 113.46 mmHg/sec for the control group and 1,043 ± 256.94 mmHg/sec for the experimental group. Although this represents a 13% decrease in dP/dt_{max} for the intermittent cardioplegia group when compared to the control group, this difference was not statistically significant.

Ventricular Weight

The ventricular weight of the hearts ranged from 3.85 g to 4.89 g, and the difference between each group's average was not significant. Ventricular weight comparison is important because the same cardioplegia dose was used for all of the hearts and any difference in weight might indicate insufficient dose in larger ventricles.

Table 2. Ventricular myocardial glycogen concentration of rabbits undergoing intermittent blood cardioplegia infusion at 37ºC. The results of the experimental and control groups in Phases I and II, expressed in % of the wet weight.

	Control	Experimental
With no Reperfusion	0.36 ± 0.05*	0.15 ± 0.03*
(Phase I)	(n=5)	(n=10)
With Reperfusion	0.24 ± 0.08	0.12 ± 0.04
(Phase II)	(n=7)	(n=10)

*p<0.01

DISCUSSION

When coronary blood flow is significantly impaired, enzymatic activity via the glycolytic mechanism (glycolysis and glycogenolysis) is inhibited resulting in lactate accumulation and intracellular acidosis [Hillis 1977, Owen 1990, Opie 1990, Opie 1992]. According to Opie there is "ATP physical compartmentalization" between the mitochondria and the cytoplasm. In functional compartmentalization, the ATP produced from glucose is used to preserve cellular membrane integrity and function, especially that of the potassium channels [Weis 1985, Opie 1992]. The ATP produced from the mitochondrial oxidative mechanism is used to support contractile functions.

In our investigation, asystole was induced aerobically by normothermic blood cardioplegia infusion. Energy reserves, especially glycogen, were relatively maintained as opposed to what would have occurred in the situation where asystole was induced by ischemia or deoxygenated cardioplegia. Therefore, the myocardium was able to tolerate the subsequent period of ischemia, being sustained by anaerobic glycolysis. Periodic infusions re-oxygenated and replaced the lost substrates consumed during ischemia and also removed the harmful products of ischemic metabolism, especially the acid radicals. Although intracellular acidosis is considered harmful, according to Rouslin it induces ATP hydrolysis into ADP and inorganic phosphate by mitochondrial ATPase [Rouslin 1983].

The results of myocardial glycogen levels in our experimental group allow us to speculate that the reserves were maintained as a result of asystolic induction prior to the ischemic period and the intermittent supply of oxygen and glucose. Another possibility would be glycolysis and glycogenolysis inhibition during the ischemic periods secondary to the accumulation of anaerobic metabolites, especially lactate and acid radicals, which inhibit glycogen consumption. However, it seems the first hypothesis is more likely.

Table 3. Mitochondrial respiration parameters (stage III and IV, RCR and ADP:0_2) in ventricular myocardium of rabbits undergoing intermittent blood cardioplegia infusion at 37ºC. Experimental and control group results in phases I and II expressed in oxygen monoatoms/mg proteinuria/minute for stage III and IV.

	PHASE I		PHASE II	
	Control Group	Experimental Group	Control Group	Experimental Group
Stage III	103.70±20.98 (n=5)	82.35 ± 9.50 (n=10)	98.54 ± 15.39 (n=7)	104.50 ± 11.89 (n=10)
Stage IV	17.16 ±4.36 (n=5)	11.04 ± 0.91 (n=10)	15.35 ± 2.86 (n=7)	16.99 ± 2.50 (n=10)
Respiratory Control Rate (RCR)	7.30 ± 0.78 (n=7)	6.38 ± 0.45 (n=10)	7.30 ± 0.78 (n=7)	6.38 ± 0.45 (n=10)
ADP/O_2 Ratio	1.99 ± 0.18 (n=5)	2.43 ± 0.22 (n=10)	2.17 ± 0.19 (n=7)	2.08 ± 0.14 (n=10)

Our results confirmed excellent functional recovery of all of the hearts. Only two hearts were discarded from the study due to ventricular fibrillation during reperfusion and air emboli were evident in the coronary arteries in both cases. Our results are similar to previous publications on the role of glucose-produced ATP.

It should also be stressed that Apstein et. al. observed severe and persistent ischemic contracture and poor functional recovery when glycolysis was inhibited by iodoacetate in hearts undergoing only 3 minutes of normothermic cardioplegia [Apstein 1978]. It should be remembered that our dosages were not carried out in the same hearts prior to and after reperfusion. It may only be concluded that at the end of the 60-minute intermittent infusion period, glycogen levels were similar to those of the group without reperfusion exposed to the same experimental conditions and that reperfusion allowed partial recovery of myocardial glycogen levels. The lower glycogen levels in the hearts of the group with reperfusion, compared to the groups without reperfusion, may be explained by the fact that at the end of the reperfusion period the ventricles were excised and immersed in cold saline solution while still in electromechanical activity. A few seconds went by before asystole was induced by ischemia and hypothermia and therefore some glycogen was consumed.

There is no consensus as to the biochemical marker that best correlates with functional changes and that has a predictive value of functional recovery after myocardial ischemia. Some authors have observed that the decrease in myocardial oxygen consumption [Zimmer 1980, Marshal 1988], and/or mitochondrial ability to produce ATP [Roenkranz 1986], were directly related to myocardial functional recovery after ischemia, although such correlations were not observed by other authors [Edoute 1983, Saks 1989]. This may be due to methodological differences in each study, especially in experimental models, ischemia, and myocardial protection protocols, and methods used to obtain the mitochondrial fraction.

Our results show a mild decrease of oxygen consumption in Stage III of mitochondrial respiration in the ischemic myocardium during the phase prior to reperfusion, although these differences were not statistically significant. In the reperfusion phase, the values were similar to control groups and again the differences were not statistically significant. The same was observed for the remaining mitochondrial respiration rates, especially RCR, which is considered to be the best indicator of mitochondrial function.

However, it should be taken into consideration that the technique we used to obtain mitochondrial fraction is very traumatic, as shown by Schlafer et al. [Schlafer 1981]. Our results may correspond only to a small amount of mitochondria that were able to survive the isolation process, usually those in better condition, underestimating the effects of ischemia on mitochondrial function. Therefore, the values obtained suggest that the infusion of normothermic blood cardioplegia every 20 minutes under these experimental conditions was effective in preserving mitochondrial function.

In regards to functional recovery, our results demonstrated that this myocardial protection technique was also effective in preserving left ventricular function. The observations by Saks et al. that the impairment of other subcellular systems, above and beyond the effects on mitochondria, are responsible for the decline in ventricular function after ischemia should also be taken into consideration [Saks 1989]. Again, the observations by Schlafer et al. about the traumatic process for obtaining mitochondrial fraction are relevant and may explain a lack of correlation between mitochondrial function and the functional recovery of the left ventricle after ischemia [Schlafer 1981].

Although continuous normothermic blood cardioplegia infusion has become increasingly popular, it should be noted that the original description of this technique by Lichtenstein et. al. was based only on theoretical considerations with no experimental evidence [Lichtenstein 1991, Lichtenstein 1992]. Therefore, there are several questions which need to be clarified. One that has concerned some investigators is the period of time during which the myocardium can tolerate interruptions of the continuous normothermic cardioplegia infusion.

Experimental studies have shown such interruptions are harmful to the myocardium [Ko 1993, Misare 1992, Matsuura 1993]. However, clinical studies have shown intermittent normothermic blood cardioplegia infusion is as effective, or even more effective than traditional intermittent hypothermic cardioplegia infusion [Pelletier 1994, Calafiore 1995, Mezzetti 1995]. Again, methodological differences should be considered to explain such contradictions.

It should be reminded that in the publications mentioned above, the intervals between infusions of intermittent normothermic blood cardioplegia or the interruption of the continuous infusion were never greater than 15 minutes. In the present investigation, the infusion of each dose was carried out for a 2-minute duration every 20 minutes. Therefore, during the 60-minute asystolic period, the hearts remained unperfused and ischemic for 52 minutes (86.6% of the time) with no apparent metabolic and/or functional consequences. However, it should be taken into consideration that these experiments were carried out in normal rabbit hearts. The results may be different in the impaired human heart, especially those with obstructive coronary disease and/or myocardial hypertrophy undergoing longer ischemic periods.

In conclusion, intermittent anterograde blood cardioplegia at 37°C for 2 minutes every 20 minutes over one hour was effective in preserving mitochondrial function and left ventricular function with partial preservation of myocardial glycogen reserves. Our results conclude that intermittant anterograde blood cardioplegia is an effective method of myocardial protection in normal rabbit hearts.

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