Crystalloid Cardioplegia Versus Oxygenated Crystalloid Cardioplegia in the Isolated Rat Heart

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Abstract

To determine if the protection afforded by a crystalloid cardioplegic solution could be enhanced by the addition of oxygen, 12 rat hearts were studied using an isolated perfused heart model, with a 2-hour ischemic arrest at 20°C.

Group 1 hearts (n = 6) received a crystalloid cardioplegic solution every 30 minutes of the arrest. Group 2 (n = 6) was treated identically except that the cardioplegic solution was oxygenated. Pre-arrest (control) and post-arrest coronary effluent enzyme levels, heart rate and coronary vascular resistance were determined. In addition time to spontaneous defibrillation was measured.

Control coronary effluent lactate dehydrogenase was elevated in group 2 ($p<0.05$). There was no significant difference in the other control parameters.

Post-arrest coronary effluent creatine kinase in group 1 and group 2 hearts was 640.67 ± 263.23 and 62.9 ± 43.83 (± SEM) ($p<0.05$ t-test). The post-arrest heart rate was 200.6 ± 13.8 and 245.5 ± 13.5 ($p<0.05$). One heart failed to spontaneously defibrillate and one had an irregular rhythm, both were in group 1. Time to spontaneous defibrillation was 58.4 ± 5.71 for group 1 and 44.67 ± 2.7 for group 2 ($p<0.05$). Post-arrest coronary effluent lactate dehydrogenase was 442.32 ± 180.74 and 100.07 ± 32.08 and showed no significant difference ($p = 0.06$).

This study demonstrates that at 20°C, markers of ischemic injury are significantly improved, leading us to conclude that the addition of oxygen to the cardioplegic solution may enhance its myocardial protective effect.

Introduction

The combination of hypothermia and potassium cardioplegic solution has been shown to be an effective method of reducing myocardial energy requirements during elective cardiac arrest.[1-8] However, it has been noted that stores of adenosine triphosphate (ATP) and creatine phosphate (CP) are gradually depleted during the ischemic episode, despite mechanical arrest of the heart and hypothermia.[3,9]

It has been proposed[10] that the reduction in ATP and CP levels is sufficient to induce failure of homeostatic mechanisms, and therefore induce myocardial damage.

These observations lead to the conclusion that anaerobic mechanisms are not able to meet the basal metabolic demands of the hypothermic arrested heart over an extended period.

A possible solution to this problem would be the intermittent regeneration of pools of ATP and CP via oxidative phosphorylation. In an attempt to achieve this, blood, crystalloid solutions and perfluorocarbons have been used as an oxygen carrying delivery vehicle for the active cardioplegic constituents.[4,11-16]

This study was designed to assess whether improved myocardial protection could be achieved by the oxygenation of a multiple dose crystalloid cardioplegic solution in the isolated rat heart.

Materials and Methods

The model used was essentially a modified form of that described by Langendorff[17] of a non-recirculating, isolated, retro-perfused rat heart, with the perfusate gravity feed system being replaced by a peristaltic pump, which may deliver a set flow in the face of a rising coronary vascular resistance. In addition the whole system is water-jacketed in order to maintain a set temperature.

12 male Wistar rats of the weight range 350–450 grams were anesthetised with chloroform in an oxygen chamber. Once anesthesia was achieved, sodium heparin was injected i.p. (300 iu), and the heart and lungs rapidly excised.

The excised heart and lungs were placed in 4°C oxygenated Krebs-Heinslet bicarbonate buffer solu-

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tion (KH). The aorta was cannulated and Langendorff perfusion with oxygenated, normothermic KH solution instituted at a flow rate of 10ml/minute. Once perfusion was initiated the lungs were removed to allow ejection of the perfusate via the pulmonary artery.

Perfusion Sequence

All hearts were subjected to the same perfusion protocol, with oxygenation of the cardioplegic solution being the only variable. Hearts were randomly allocated to the unoxygenated group (group 1), or the oxygenated group (group 2), by a microcomputer.

5 minutes were allowed for stabilization of the heart. There then followed a 10 minute control period, during which the heart rate (HR) and coronary vascular resistance (CVR) were determined. Also, coronary effluent was collected for subsequent analysis of the enzymes lactate dehydrogenase (LD) and creatine phosphokinase (CK).

At the end of the control period the hearts were made globally ischemic and 20°C unoxygenated cardioplegic solution (CSU) infused into the aortic root, at a pressure of 60 cm.H_2O for 3 minutes. In addition the water jacket around the heart was set to 20°C. The temperature of 20°C was chosen as this is our upper clinically acceptable temperature for ischemic arrest.

Subsequent infusions of either CSU or oxygenated cardioplegic solution (CSO) were made at 30 minute intervals, again at 60 cm.H_2O pressure for 3 minutes. At the end of 2 hours ischemic arrest reperfusion was instituted with normothermic oxygenated KH solution.

A 5 minute period (RP1) was allowed for stabilization of the heart. During this time HR and CVR were determined, and coronary effluent collected for LD and CK leakage determination. The time taken to spontaneous defibrillation was noted. Any hearts failing to spontaneously defibrillate within the 5 minutes were electrically defibrillated with a DC shock.

During the final 10 minute perfusion period (RP2) HR and CVR were determined, and coronary effluent collected. At termination of the experiment the heart was dried at 100°C for 24 hours for determination of heart dry weight.

Perfusates

The composition of the perfusate KH by weight in grams per liter was as follows; NaCl-6.90, KCl-10.35, $CaCl_2.6H_2O$-0.56, $NaHCO_3$-2.10, KH_2PO_4-0.16, $MgSO_4.7H_2O$-0.29, Glucose-2.0. The pH of the solution in group 1 was 7.396 ± 0.0146 (± SEM) and 7.418 ± 0.0053 respectively (t-test not significant NS). The pO_2 in kilo pascals was 74.815 ± 2.921 and 80.235 ± 2.472 (NS), and the pCO_2 4.57 ± 0.169 and 4.585 ± 0.103 respectively (NS).

The cardioplegic solution was essentially a type 1 St. Thomas', either oxygenated with 95% oxygen / 5% carbon dioxide, or unoxygenated and buffered to pH 7.65 at 20°C. The constituents and gases of the cardioplegic solutions were as follows; K^+ 20 mMol/1, Na^+ 147.2 mMol/1, Mg^{2+} 16 mMol/1, Cl^- 204.6 mMol/1, Ca^{2+} 2.2 mMol/1, procaine HCl 273 mg/1, 50% glucose 20 ml/1. Group 1 pO_2 was 11.87 ± 1.107 and group 2 84.64 ± 5.055 ($p<0.05$). The pCO_2 for the two groups was 0.798 ± 0.046 and 0.634 ± 0.059 ($p<0.05$).

Statistics

All data were analyzed using a BBC microcomputer with Unistat (University Software) statistical analysis package. In all cases the mean of one group was compared to the mean of the other group using a two sample t-test, with either even or uneven variance as determined by a f-test.

In the case of enzyme leakage, results were corrected for heart dry weight prior to analysis.

Heart Rate Determination

Heart rate was determined from hard copy recordings of the ECG. In the case of the control period and the RP2 period this consisted of an average of three 20 second recordings—at the beginning of the period, midway through the period and again at the end. In the case of the first 5 minutes of reperfusion (RP1) the heart rate was determined from a single 1 minute recording, at the end of the period.

Coronary Vascular Resistance

As this isolated retroperfused rat heart model is of the fixed flow type, any variation in perfusion pressure will be directly proportional to changes in coronary vascular resistance. In this study CVR is expressed as the perfusion pressure in mmHg. and is an average of three readings. The readings were taken at the start of the period, midway through the period and at the end.

Lactate Dehydrogenase Leakage

Coronary effluent was analyzed for the cytosolic enzyme lactate dehydrogenase (LD) according to the method of Wroblewski and La Due.[18] LD was measured using a quantitative kinetic assay kit (340-UV)[a] and a spectrophotometer, SP30UV.[b]

Coronary effluent samples were assayed within 30 minutes of collection and control values checked from Precipath U control sera.[c] The assays were performed

a Sigma Chemical Company, Dorset, England BH17 7NH
b Pye Unicam.
c BCL, Sussex, England BN7 1LG

Table 1.
Heart Rate (bpm)

	Control		RP1		RP2	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
	171	230	VF*	259	143	207
	263	294	235	300	199	281
	314	281	251	305	224	287
	262	263	206	201	225	215
	311	255	235	226	212	238
	252	232	Irreg**	192	Irreg**	245
Mean	262.2	259.2	231.8	247.2	200.6	245.5
SEM	21.2	10.5	7.7	19.2	13.8	13.5
t-test	NS		NS		$p < 0.05$	

* Ventricular fibrillation
**Irregular rhythm

at 25°C and results converted to international units per liter, then adjusted for heart dry weight.

Creatine Phosphokinase Leakage

Coronary effluent was analyzed for the membrane bound enzyme creatine phosphokinase (CK) according to the methods of Oliver[19] and Rosalki.[20]

CK was measured with a quantitative kinetic enzyme assay kit (45-UV) supplied by the Sigma Chemical Company, using a spectrophotometer (SP3OUV).

Coronary effluent samples were analyzed within 30 minutes of collection and control values checked from CPK1 rabbit serum supplied by the Sigma Chemical Company.

The assays were performed at 25°C and results converted to international units per liter, then adjusted for heart dry weight.

Results

Table 1 shows the recorded heart rate for each heart in each of the three sample periods, (control, RP1 and RP2). One heart failed to spontaneously defibrillate and one heart suffered an irregular rhythm post ischemia. Both were in the unoxygenated cardioplegia group (group1). For the purposes of the determination of the mean these were excluded from the results for the relevant period.

As can be seen group 1 hearts (CSU) demonstrated a steady reduction in heart rate (Figure 1) with group 2 hearts (CSO) showing a statistically significant maintenance of the heart rate.

The assessment of coronary vascular resistance (Table 2), although previously reported to be a good indicator

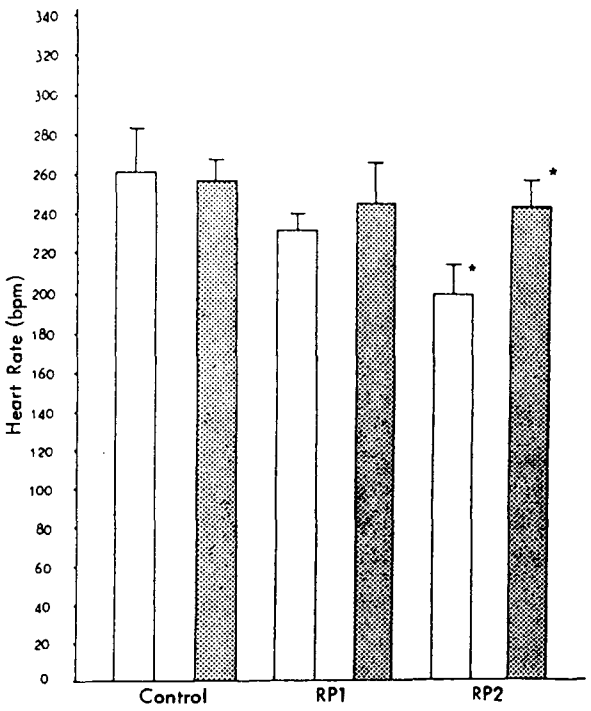

Figure 1. Creatine Phosphokinase Leakage (* $p < 0.05$ between two marked groups, ** $p<0.05$ between two marked groups).

Table 2.

Coronary Vascular Resistance (mmHg)

	Control		RP2	
	Group 1	Group 2	Group 1	Group 2
	59	64.5	69	71
	63	54	93.5	74
	64.5	60.5	132	66.5
	59.5	59	64.5	65
	54	62.5	64	71.5
	59.5	65	99	67
Mean	59.9	60.9	87	69.2
SEM	1.49	1.67	10.91	1.43
t-test	NS		NS (p = 0.08)	

of myocardial damage,[21] did not demonstrate statistical significance (p = 0.08) in the post-arrest RP2 period.

Table 3 and Figure 2 show the results for lactate dehydrogenase leakage. Unfortunately we were unable to demonstrate statistically significant differences in the RP2 period between the two groups, (p = 0.06). Of note is that the control LD in group 2 was significantly elevated when compared to group 1.

The creatine phosphokinase leakage (Table 4, Figure 3) showed no difference in the control period. Statistical significance was observed in both post-arrest periods (RP1 and RP2) with less enzyme leakage in the oxygenated cardioplegia group (group 2).

Table 5 shows the time taken from institution of reperfusion to spontaneous defibrillation. The one heart that failed to spontaneously defibrillate has been excluded from the results. These results demonstrate that group 2 hearts defibrillated significantly quicker than group 1 hearts.

Table 3.

Lactate dehydrogenase leakage (iu/l/g heart dry weight)

	Control		RP1		RP2	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
	35.92	63.32	1108.06	83.98	1023.08	40.43
	13.92	9.99	261.05	29.99	522.1	209.94
	21.17	33	74.08	90.76	42.33	90.76
	14.65	89.62	109.94	100.82	87.85	67.21
	6.97	39.96	101.08	79.92	73.2	179.82
	0	32.65	562.81	36.73	905.38	12.24
Mean	15.44	45.09	369.5	70.37	442.32	100.07
SEM	5.05	11.48	165.46	12.08	180.74	32.08
t-test	p <0.05		NS (p = 0.065)		NS (p = 0.06)	

Table 4.

Creatine phosphokinase leakage (iu/l/g heart dry weight)

	Control		RP1		RP2	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
	0	6.22	1111.23	0	1349.94	0
	38.92	0	302.82	33.32	870.17	276.59
	0	11	0	19.25	0	19.25
	0	26.14	271.2	67.21	135.6	14.94
	0	0	121.99	26.64	97.6	66.6
	8.16	0	709.62	0	1390.78	0
Mean	7.74	7.23	419.48	26.07	640.67	62.9
SEM	6.25	4.2	169.58	10.19	263.23	43.83
t-test	NS		$p < 0.05$		$p < 0.05$	

Conclusions

The hypothesis that technique 1 (CSU) does not offer as great a degree of protection to the ischemic myocardium as technique 2 (CSO) is well supported by the results. In particular the fact that the only heart

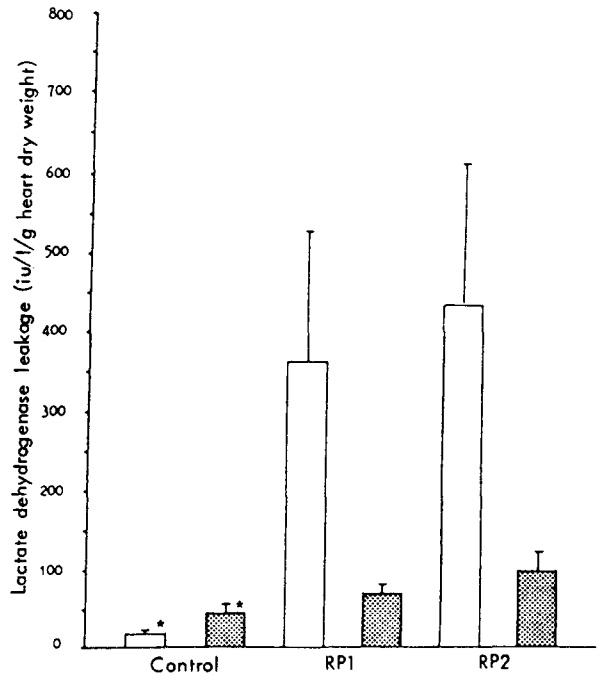

Figure 2. Lactate Dehydrogenase Leakage (* $p<0.05$ between two marked groups).

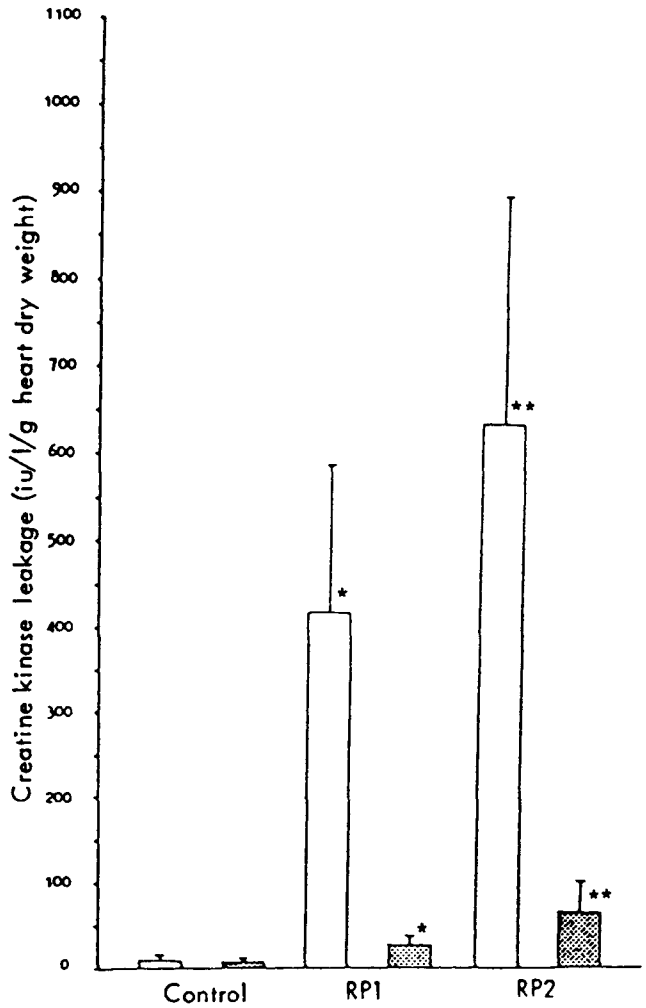

Figure 3. Heart Rate (*$p<0.05$ between two marked groups)

Table 5.

Time to spontaneous defibrillation (seconds)

	Group 1	Group 2
	– *	55
	72	35
	45	41
	50	45
	50	45
	75	47
Mean	58.4	44.67
SEM	5.71	2.7
t-test	$p < 0.05$	

* Did not spontaneously defibrillate

to suffer a rhythm disturbance, and the only heart not to spontaneously defibrillate were both in group 1 (CSU) is of note.

With respect to the enzyme leakage, it may be that both techniques allowed a level of damage sufficient to make the cell membranes "leaky" thus allowing escape of free cystolic enzymes such as LD, but the degree of damage of group 1 hearts was of sufficient magnitude to allow a greater release of membrane bound enzymes such as CK.

The mechanism by which the oxygenated cardioplegia may produce improved myocardial protection is in the regeneration of high energy phosphate pools (ATP and CP). Much ischemic myocardial damage has been associated with the energy depletion induced failure of homeostatic mechanisms, and in particular the mechanisms responsible for maintaining calcium and sodium homeostatis. Intermittent regeneration of ATP and CP would allow homeostatic mechanisms to function during the ischemic episode.

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Questions from the Audience

Al Stammers, Ann Arbor, MI: Question: Have you done any functionality studies post-ischemia, and if so, do these reflect the information you have shown with your enzyme passes?
Answer: Not in this particular phase of the experiment. Previously, functional studies have correlated with enzyme leakage. There is an excellent correlation between the leakage of enzymes on functional studies, both with simple return of the amount of the aortic pressure that can be developed by the left ventricle and also with codes. It was a bit disappointing that there was no significant difference in the lactate dehydrogenase post-ischemia. Possibly worth nothing, I was recently reading in a statistical book only last week, which stated that a non-significant result doesn't mean that the number is not of significance. This could be seen from the graph. I think primarily there is a variance of the sump post, which was leading to a non-significant result in the lactate dehydrogenase leakage.
Chuck Dyson, UCLA: Question: In your experimental design, you had 10 minutes for the hearts to recalibrate or come back to baseline, are you sure that was enough time and still not leaking enzymes from your surgical press? If your heart was still coming down to baseline, then you could assume, you would accept an abnormally high baseline and your damaged sample would be narrowed so you would assume you are not doing as much damage as you are if, in fact, the heart would not come back to the baseline. Also, did you look at any electron-micrographs of the heart muscles after they had been reperfused to see if there were any changes or improvements in the myocardial protection with oxygen versus non-oxygen?
Answer: Whether the stabilization time was long enough for many previous experiments along with similar lines with this length of time doesn't appear to be long enough; and foremost, the literature did not find the wide variety of stabilization times. But you know in many cases, this appears to be associated with what you want to achieve in that time. If you go into the functional studies, then the literature shows that the stabilization time is prolonged, this is primarily because it takes longer time to derive the control parameters and this isn't because the heart doesn't become stable. It is just the time was necessary to do certain other procedures.