

EVALUATION OF SANGUINEOUS AND CRYSTALLOID CARDIOPLEGIC SOLUTIONS DURING TOTAL HEART-LUNG BYPASS IN DOGS

R.S. George¹, R. Jayaprakash², K. Ramanujam, C. Radhakrishnan and D. Archibald

¹ Department of Clinical Studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia.

² Department of Clinics, Directorate of Clinics, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India.

SUMMARY

Twenty-four mongrel dogs were subjected to sanguineous and crystalloid cardioplegia for a period of thirty minutes at 20°C and 25°C systemic hypothermia during cardiopulmonary bypass. Cardioplegic solutions were administered at 4°C to induce cardioplegia. Physiological functions of the myocardium revealed that the cardiac function returned to normal sinus rhythm without any loss when blood cardioplegic solution was used at both hypothermic temperatures. The time taken for cardioplegia and cardiac electrical quiescence was achieved earlier at 20°C. Animals in sanguineous cardioplegic group revealed better functional return and revival of cardiac musculature in terms of lesser applications of defibrillator, lesser requirement of inotropic support and early cardiac contraction. This study revealed that sanguineous cardioplegic solution at a systemic temperature of 20°C was found to afford better myocardial protection during an arrest period of 30 minutes.

Keywords: Cardioplegia, hypothermia, cardiopulmonary bypass

INTRODUCTION

Myocardial ischemic arrest is an essential requirement during intra-cardiac surgical procedures. Myocardial preservation during ischemic arrest has been achieved by topical cooling of the heart musculature or by the administration of cardioplegic solutions, wherein the metabolic demands are lowered. The two most popular methods of pharmacological arrest utilise either blood or crystalloid solutions as a delivery vehicle. (Axford-Gatley *et al.*, 1990; Coetzee *et al.*, 1990). Despite the advances in the composition of the cardioplegic solutions, the temperature at which it should be delivered and the method and frequency of administration, a significant percentage of patients demonstrate clinical evidence of myocardial damage in the post-operative period.

This study was conducted to compare crystalloid and sanguineous cardioplegic solutions using identical potassium concentrations, identical methods of administration and identical temperatures of delivery at two systemic hypothermic temperatures. The objective was to study the effectiveness of blood and crystalloid cardioplegic solutions at two different hypothermic temperatures, the physiologic, haemodynamic and biochemical changes before, during and after cardioplegic status and assess the extent of myocardial protection afforded by the cardioplegic solutions by histopathology.

MATERIALS AND METHODS

Twenty four mongrel dogs maintained under identical feeding and management were used for the study. The dogs were grouped as follows:

Grp	Subgrp	Treatment	
		Hypothermic temperature	Cardioplegic solution used
I	A	20°C	Sanguineous
	B	25°C	Sanguineous
II	A	20°C	Crystalloid
	B	25°C	Crystalloid

Preparation of animals

The animals were premedicated with atropine sulphate (0.05mg/kg) s/c and acepromazine (0.1mg/kg) i/m. Anaesthesia was induced with 2.5% thiopentone sodium (12.5mg/kg) and maintained with isoflurane. Muscle relaxant pancuronium bromide (0.1mg/kg) i/v was administered before connecting the animal to the ventilator. Ventilation was maintained at a tidal volume of 15ml/kg and the rate was adjusted to 12 breaths/min.

Blood was collected from the donor after cross matching with the patient for compatibility.

Preparation of cardioplegic solution

Sanguineous cardioplegic solution was prepared by obtaining 500ml of blood from the pump oxygenator to which 30mEq/L of potassium chloride was added. Crystalloid cardioplegic solution was prepared by addition of 30mEq/L potassium chloride, 1g of methyl prednisolone and 16ml of 50% dextrose to 1L of Ringer's solution. The pH of the cardioplegic solutions were adjusted to a pH of 8 by addition of sodium bicarbonate solution. The cardioplegic solutions were cooled to 4°C. (Catinella *et al.*, 1984a).

Priming the bypass circuit

A DIDEKO disposable pediatric bubble oxygenator with heat exchanger and pump was primed with 1300ml of lactated Ringers solution and 700ml of blood from the donor to maintain a haematocrit value of 25%. Sodium bicarbonate was added to maintain a pH of 7.35-7.45. Heparin at a total dose of 4000 units and mannitol at 5mg/kg were also added to the priming solution.

Surgical procedure

The heart was approached through a midsternal incision. Cannulas were placed in the anterior vena cava, posterior vena cava and the aorta. The animal was then heparinised at a dose rate of 5mg/kg before institution of cardiopulmonary bypass.

The aortic line from the pump was connected to the aortic cannula and the venous cannulas to the venous drainage port of the bubble oxygenator. Perfusion was maintained to achieve a mean arterial pressure of 70-85mm of Hg and the flow was adjusted to maintain a flow rate of 70-90ml/kg/min. Systemic hypothermia of 20 °C or 25°C was achieved by circulating chilled water into the oxygenator. (Ellis *et al.*, 1979; Khuri *et al.*, 1988). The systemic core temperature was monitored by an oesophageal temperature probe placed at the level of the cardia.

On reaching a core temperature of 20°C and 25°C, cardioplegia was initiated after cross clamping the aorta and injecting the cold cardioplegic solutions through a 12 G cardioplegic cannula placed at the aortic root. To achieve 30 minutes of ischemic arrest, the cardioplegic solutions were administered at a rate of 10ml/kg over a period of 2-3 minutes. Supplemental doses were administered as and when electrical activity was observed. (Baraka *et al.*, 1993). The cardiac arrest period at 20°C or 25°C systemic hypothermia using sanguineous or crystalloid cardioplegic solutions for 30 minutes was maintained as a constant factor during the study.

Rewarming and reperfusion was initiated in both the groups after 30 minutes of cardioplegia by releasing the aortic cross clamp and raising the temperature of the circulating blood in the oxygenator to 42°C so as to

achieve a systemic temperature of 38°C. Functional revival of the myocardium was spontaneous after rewarming and reperfusion and was assisted by defibrillation as and when required. (Axford-Gatley *et al.*, 1990).

Parameters studied

I. Physiological-cardiac function

Heart rate was recorded before thoracotomy, during open thorax, before cardioplegia, before and 15 minutes after release of aortic cross clamp.

- i) Time for cardioplegia: the time taken in minutes for cardiac arrest from the time of delivery of cardioplegic solution.
- ii) Time for cardiac electrical quiescence: the duration in seconds to achieve nil electrical activity after administration of cardioplegic solutions.
- iii) Return and revival of cardiac function
 - a) Time for appearance of ventricular fibrillation: the time in seconds taken for appearance of ventricular fibrillation as the cardioplegic effect waned off and the systemic temperature increased.
 - b) Functional return on rewarming and reperfusion: the number of hearts that returned to normal sinus rhythm and the number of hearts that fibrillated during rewarming and reperfusion.
 - c) Number of applications of defibrillator: the number of applications of defibrillator on the fibrillating hearts.
 - d) Inotropic support: the number of hearts requiring inotropic support after cross clamp removal.
- iv) Time for cardiac contraction: the time in minutes taken for the appearance of normal cardiac contractions after cardioplegia.
- v) Time for sinus rhythm: The time taken, in minutes, for appearance of normal sinus rhythm.
- vi) Volume of cardioplegic solution delivered: the volume in milliliters of cardioplegic solutions required for 30 minutes of cardioplegia.
- vii) Electrocardiography: electrocardiographs were recorded using the standard limb leads for continuous monitoring of myocardial activity.

II. Haemodynamics

- i) Mean arterial pressure was recorded through the femoral artery cannulation and central venous pressure was recorded through jugular vein cannulation before thoracotomy, at open thorax and 15 minutes after release of aortic cross clamp.

- ii) Left Ventricular Pressure: systolic and diastolic pressure of the left ventricle was recorded through a cannula placed in the left ventricle at open thorax, before bypass, after resumption of sinus rhythm and 15 minutes after reperfusion.
- iii) Coronary sinus effluent recovered and flow rate: coronary sinus effluent was collected through a cannula placed in the right atrium from the time of delivery of cardioplegic solution till the end of cardioplegia.

The coronary effluent flow rate was determined as per the formula:

$$\text{CEFR} = \frac{\text{Coronary effluent (in ml)}}{\text{Min. of cardioplegia}} \times \frac{100}{\text{Wet wt of heart}}$$

III. Biochemical

- i) Systemic
Partial pressure of oxygen and carbon dioxide tension and serum enzymes – creatine phosphokinase, lactate dehydrogenase and aspartate amino transferase were estimated before thoracotomy, after thoracotomy, during bypass and 15 minutes after reperfusion.
- ii) Coronary
Partial pressure of oxygen and carbon dioxide tension and coronary enzymes - creatine phosphokinase, creatine kinase isoenzyme-MB and lactate were estimated at 15th and 30th minute of cardioplegia.

IV. Myocardial Water Content

Myocardial water content was estimated by harvesting the hearts after 15 minutes of reperfusion by the formula:

$$\frac{\text{Wet Wt} - \text{Dry Wt}}{\text{Wet Wt}} \times 100$$

V. Histopathology

Transmural sections of left ventricle fixed in 10% buffered formalin and stained with hematoxylin and eosin were examined for presence of markers of myocyte damage such as contraction band necrosis, hyper-eosinophilia or smudging of myofibrils with loss of cytoplasmic details. The data were subjected to equal or unequal completely randomised design and analysed statistically.

RESULTS AND DISCUSSION

Physiological-cardiac function

Irrespective of the systemic hypothermic temperatures, the heart rate in all the animals in Group I returned to normal sinus rhythm 15 minutes after release

of aortic cross clamp, whereas only four hearts at 20°C and five hearts at 25°C systemic hypothermia returned to functional status in Group II. The better functional return of hearts in the sanguineous group can be attributed to the presence of red blood cells which protected the myocardium against reperfusion injury by increased oxygen delivery and the presence of protein which buffered the oxygen free radical metabolites (Van Asbeck *et al.*, 1975).

The mean time taken to achieve cardioplegia in seconds was 15.00±0.83, 19.04±0.78, 18.60±1.09 and 21.18±0.73 in subgroups A and B in Groups I and II respectively. Statistical analysis indicated a decrease in the time taken for cardioplegia in subgroup A in Groups I and II and may be due to lower systemic hypothermia resulting in lowered metabolic demands of the hearts (Maloney and Nelson, 1975; Standeven *et al.*, 1979).

Cardiac electrical quiescence was achieved at a shorter period of time with 20°C systemic hypothermia. The average time in seconds was 18.61±0.73, 21.00±0.76, 20.24±0.77 and 24.03±1.09 in subgroups A and B in Groups I and II respectively. The quicker electrical quiescence at 20°C in both groups and with sanguineous cardioplegia may be due to the presence of intrinsic potassium in the blood cardioplegic solution which altered the movement of intracellular potassium and thereby altering the electrical gradient across the cell membrane (Surawicz, 1980; Cunningham *et al.*, 1979).

The time for appearance of ventricular fibrillation was significantly lesser at 20°C hypothermia in both the groups. Five hearts and three hearts in subgroup A and B of Group I returned spontaneously to functional mode while only two hearts in subgroup A and one heart in subgroup B in Group II were functional. Though the number of applications of the defibrillator was not statistically significant between the groups, it was lesser in Group I. Inotropic support was instituted after attempts to defibrillate failed. The number of animals that required inotropic support was 1, 0, 3 and 3 in subgroups A and B of Group I and II respectively. The time taken for cardiac contraction to appear after rewarming showed no significant change in either groups whereas, the time taken for sinus rhythm to appear was significantly lesser in Group I. The results of the study revealed better functional return and revival of cardiac musculature when sanguineous cardioplegic solution at 20°C was used in terms of lesser applications of the defibrillator, lesser requirement of inotropic support, early cardiac contraction and appearance of sinus rhythm (Cunningham *et al.*, 1979; Acar *et al.*, 1991a; 1991b; Kofsky *et al.*, 1991; Robinson and Harwood, 1991; Brown *et al.*, 1993).

The volume of cardioplegic solution required for thirty minutes of cardioplegia was 306.56±12.4, 324.29±5.11, 358.40±10.59 and 369.72±6.93 milliliters in subgroups A and B of Groups I and II respectively. Statistical analysis revealed that a significantly higher

volume of crystalloid cardioplegic solution was required for 30 minutes of ischemic arrest. The lower volume of sanguineous cardioplegic solution required maybe due to its cellular content apart from the intrinsic contents in blood that were able to meet the metabolic demands of the myocytes during ischemia (Surawicz, 1980).

Following rewarming and reperfusion, ventricular tachyarrhythmia of electrocardiographic configurations were observed. The ventricular fibrillations were frequently accompanied by ventricular extra systoles and sinus beats. In the sanguineous group, the ectopic ventricular complex was interrupted by the T wave of the preceding complex. Fifteen minutes following reperfusion, an increase in the width of the P wave and elongation of the QRS complex was evident. The P-R interval was also increased accompanied by elongated Q-T interval. Ventricular fibrillation following aortic cross clamp release may be due to hypoxia and release of catecholamines resulting in increased influx of calcium (Meesmann *et al.*, 1976; Pollock, 1977).

Haemodynamics

The mean arterial pressure revealed a significant decrease during open thorax and was comparable with the mean pressure after release of aortic cross clamp. The mean arterial pressure after 15 minutes of reperfusion at 20°C in the blood cardioplegia group improved indicating better maintenance of cardiac contractility, preservation of high energy phosphates and decreased lactic acid accumulation during the anaerobic arrest. The findings concurred with the findings of Coetzee *et al.*, 1990; Shragge *et al.*, 1978; Panos *et al.*, 1990.

The central venous pressure was elevated at open thorax when compared to the base values. At 15 minutes after reperfusion, the pressure was significantly higher than at open thorax in both the groups. The elevation in central venous pressure after release of aortic cross clamp release may be due to over-hydration during bypass. (Kofsky *et al.*, 1991).

The mean left ventricular systolic and diastolic pressures showed an insignificant increase at both the hypothermic temperatures when sanguineous cardioplegia was used. Sanguineous cardioplegia afforded greater protection to the myocardium by preventing ischemia and infarction and providing increased capillary blood flow, maintaining oxygen demand-supply ratio and thereby providing energy to the reviving myocardial cells (Ko *et al.*, 1992).

The mean coronary effluent flow rate was 5.32 ± 0.34 , 5.67 ± 0.35 , 6.95 ± 0.41 and 6.98 ± 0.32 milliliters per 100G of heart per minute in subgroups A and B of Groups I and II respectively. Statistical analysis revealed a higher coronary effluent flow rate with crystalloid cardioplegic solution when compared to sanguineous cardioplegic solution. The increased flow rate in the crystalloid group

can be attributed to the reactive hyperemia which followed ischemia and the lower flow rate with sanguineous cardioplegic solution to increased viscosity of blood at lower temperatures and the high coronary vascular resistance during hypothermia (O'Neill *et al.*, 198; Catinella *et al.*, 1984). Crystalloid cardioplegia at 25°C hypothermia revealed higher retention because of lower vascular resistance (Acar *et al.*, 1991) damage to the endothelial lining (Saldhana and Hearse, 1989) and inadequate myocardial protection resulting in myocardial edema (Feindel *et al.*, 1984).

Biochemical

1. Systemic – blood gas and enzymes

The mean systemic partial pressure of oxygen was significantly higher in subgroups A when compared to subgroups B in Groups I and II. After reperfusion, Group I animals had a higher arterial oxygen tension than Group II animals. The partial pressure of carbondioxide also showed a significant increase after thoracotomy in both the groups. The increased carbondioxide tension during bypass may be attributed to atelectasis of alveoli and increased pulmonary hypotension. The higher arterial oxygen tension at 20°C hypothermic temperature during bypass may be due to reduced tissue metabolism and the decreased affinity of tissues to attract oxygen (Catinella *et al.*, 1984a; Axford-Gatley *et al.*, 1990).

The systemic creatine phosphokinase levels showed a significant increase during bypass and 15 minutes after reperfusion and the mean values were significantly higher in Group I than in Group II. The increase can be attributed to the tissue damage caused to skeletal muscles during thoracotomy, suboptimal oxygen utilisation and myocardial ischemia (Goto, 1974). When comparing the means of sanguineous and crystalloid cardioplegia, the levels were higher in the latter indicating lesser protection during global ischemia (Cerra *et al.*, 1975; Ahumada *et al.*, 1976).

A significant increase in the mean lactate dehydrogenase level was evident during the various stages. However, after 15 minutes of reperfusion a peak LDH value was observed in subgroup B of Group I followed by subgroup B of Group II, subgroup A of Group I and subgroup A of Group II. LDH is not specific to any tissue but is released extracellularly during cell damage. The LDH release was more pronounced at 25°C and may be due to higher oxygen demand at 25°C and decreased availability of oxygen (Brown *et al.*, 1993).

The analysis of variance showed a significant increase ($P < 0.01$) in the mean aspartate amino transferase level during bypass in all the subgroups. However, subgroup B of Groups I and II maintained significantly high enzyme levels. Higher levels of aspartate amino transferase at 25°C systemic hypothermia could be due to higher oxygen requirement than at 20°C. Thomas and

Kurt (1979) attributed this elevation to a reduction in blood flow, hypotension and transient hypoxemia.

2. Coronary – blood gas and enzymes

The mean partial pressure of oxygen in the coronary sinus effluent ranged between 290.34 ± 10.80 and 292.62 ± 13.43 in the sanguineous group and between 164.14 ± 5.95 and 165.83 ± 6.20 in the crystalloid group. The partial pressure of oxygen in the coronary sinus effluent was significantly high and the oxygen extraction by the myocardium (PO_2 of cardioplegic solution minus PO_2 of coronary effluent) was increased with sanguineous cardioplegic solution. The sanguineous cardioplegic solution contained oxygen in both the dissolved and hemoglobin-bound state and may be the reason for the elevated values (Warner *et al.*, 1987).

No significant change in the mean carbondioxide tension was evident in the two cardioplegic solution groups. However, the levels were higher in Group II at 15th and 30th minute of cardioplegia suggesting higher level of ongoing anaerobic metabolism in the myocardium (Bing *et al.*, 1982; Feindal *et al.*, 1984; Kofsky *et al.*, 1991).

The coronary effluent creatine phosphokinase leakage exhibited a significant progressive increase as the duration of ischemia advanced up to 30th minute of cardioplegia in the crystalloid cardioplegic group and is attributed to the conversion of aerobic to anaerobic metabolic pathways in the myocardial cells (Gray *et al.*, 1977). However the increase was mild in the blood cardioplegic group because of the presence of oxygen for aerobic metabolism to continue and synthesise high energy phosphates.

Creatine kinase isoenzyme MB considered as the most sensitive and specific marker of myocardial injury increased significantly in subgroup B of Group II followed by subgroup B of Group I and subgroup A of Group II. This indicates that at 25°C systemic hypothermic temperature, both the cardioplegic solutions offered lesser protection to the myocardium (Yau *et al.*, 1993).

Analysis of variance revealed a significant decrease in the mean lactate level with sanguineous solution when compared to crystalloid solution. The mean lactate level progressively increased significantly at 15th and 30th minutes with crystalloid cardioplegia. Lactate being the end product of anaerobic glycolytic metabolic pathway, was lower in the ???when blood cardioplegia was used. As the sanguineous solution provided the required quantity of oxygen during ischemia, lactate release was negligible (Warner *et al.*, 1987).

The mean percentage of myocardial water content was 78.83 ± 0.22 , 78.86 ± 0.27 , 80.34 ± 0.86 and 81.01 ± 0.37 in subgroups A and B if Groups I and II respectively. Oxygen delivery to the myocardium is comparatively lesser with crystalloid cardioplegic solution leading to an increase in the production of oxygen free radicals which triggers inflammatory response in the ischemic myocardium.

Myocardial hypoxia has also been incriminated for the higher myocardial water content. Edema of the myocardium may also be due retention of cardioplegic solution in the crystalloid group which correlated with the higher coronary sinus effluent flow rate (Tabayashi *et al.*, 1991).

Histopathology

The histopathological examination of sections of left ventricle revealed markers of myocyte damage with both the groups at 20°C and 25°C systemic hypothermia. In subgroup A of Group I, very few areas of myocyte injury could be identified. The ratio of fields exhibiting markers of myocyte damage such as hyper eosinophilia was only 30:5. In subgroup B of Group I, in eight out of thirty fields examined, mild myofibrillar smudging and eosinophilic infiltration in the myocardium could be detected. Myocardial sections in Group II animals showed hyper eosinophilic infiltration and extensive loss of striations of myofibrils. These markers of injury were at the ratio of 30:10 in subgroup A and 30:14 in subgroup B. Severe myocardial edema was also evident in all the group II hearts.

The presence of mild eosinophilic infiltration in Group I might be attributed to inflammation of the myocytes during ischemia and after reperfusion resulting from change in temperature and substrate (Brown *et al.*, 1993). The severe myocyte damage in Group II may be due to the lack of oxygen carrying capacity of the crystalloid solution (Barner, 1991; Cunningham *et al.*, 1979).

CONCLUSION

Sanguineous cardioplegia delivered at 4°C at a systemic hypothermic temperature of 20°C provided adequate myocardial protection and early return to function during total heart-lung bypass in dogs.

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