Evidence that continuous normothermic blood cardioplegia offers better myocardial protection than intermittent hypothermic cardioplegia

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Abstract

Objectives—To compare transmyocardial ischaemia and oxidative stress, as well as non-infarction myocardial injury, in patients randomised to intermittent hypothermic cardioplegia or continuous normothermic blood-potassium cardioplegia.

Design—Prospective randomised trial.

Setting—Tertiary cardiac referral centre.

Methods—24 patients undergoing elective coronary artery bypass surgery were randomised to hypothermic (13 patients, mean (SEM) age 59.5 (2.6) years) or normothermic (11 patients, mean (SEM) age 59.7 (3.3) years) cardioplegia. Transmyocardial oxidative stress and ischaemia were assessed by the difference in plasma concentrations of oxidised glutathione and lactate respectively, from samples taken simultaneously from the coronary sinus and aortic root. Blood samples were taken just before cross clamp application and at intervals up to 15 min after cross clamp release. Non-infarction myocardial injury was assessed by measurement of creatine kinase MB isoenzyme activity from peripheral venous blood taken 2 and 18 h after surgery.

Results—Intermittent hypothermic cardioplegia resulted in a significant increase in transmyocardial ischaemia ($P < 0.001$) and oxidative stress ($P < 0.001$). Evidence of significantly increased myocyte damage was also present ($P < 0.01$). No significant corresponding changes were present with normothermic cardioplegia.

Conclusions—Normothermic blood cardioplegia seems to avoid significant changes in myocardial ischaemic status and consequent oxidative stress. This study provides direct evidence that normothermic cardioplegia offers enhanced myocardial protection compared with that of hypothermic cardioplegia. Certain subsets of patients may derive more benefit from normothermic cardioplegia, although it is unclear whether this would be the case for all patients.

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Keywords: continuous normothermic cardioplegia; intermittent hypothermic cardioplegia; myocardial protection; ischaemia and oxidative stress

In 1989 a new concept in myocardial protection was initiated as an alternative to the standard use of hypothermia.¹ This technique involved continuous, normothermic blood-potassium perfusion during the cross clamp period, which aims to provide an uninterrupted supply of oxygenated blood to the myocardium. Not only has continuous normothermic cardioplegia been found to be safe and effective, but it has also been proposed that the method provides a superior means of myocardial protection over intermittent cold blood cardioplegia.²⁻⁷ No prospective study, however, has been carried out to show benefit in terms of myocardial metabolism and free radical activity.⁸

The aims of this study were to examine transmyocardial oxidative stress and ischaemia, using plasma oxidised glutathione and lactate concentrations, in patients undergoing elective coronary artery bypass surgery by two different methods of myocardial protection: (a) antegrade induction followed by intermittent retrograde hypothermic cardioplegia (group A); and (b) antegrade induction followed by continuous, retrograde normothermic blood-potassium cardioplegia (group B). In addition, differences in non-infarction myocardial injury were assessed by measurement of serum creatine kinase MB isoenzyme concentrations from peripheral venous blood taken 2 and 18 h after surgery.

Patients and methods

PATIENTS

In this prospective study comprising 24 patients, 13 were randomised to group A and 11 to group B. Randomisation was carried out according to the patient's hospital number, and all patients underwent elective coronary artery graft surgery only. The study was approved by the hospital ethical committee.

OPERATIVE TECHNIQUES

All patients underwent cardiopulmonary bypass using an ascending aortic cannula for arterial return and a two stage venous cannula inserted into the right atrium. A Cobe CML membrane oxygenator (Cobe Laboratories, Quedgeley), in line filter, and crystalloid prime were utilised. In addition to the cannulations for bypass, a coronary sinus perfusion cannula was introduced through a separate purse string suture in the right atrium and manually guided into the coronary sinus.

In group A, cardiopulmonary bypass was carried out at moderate systemic

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hypothermia, with a nasopharyngeal temperature of 28°C. Cardiac arrest was induced after aortic cross clamping with an antero-grade infusion of blood cardioplegic solution (600 ml) into the aortic root (composition: potassium chloride 20 mmol/l for initial dose, 10 mmol/l for repeat doses; calcium 0.6 mmol/l; glucose 225 mmol/l; osmolality 320–360 mmol/kg; pH 7.8–8.0 (THAM); haematocrit 20–25%; temperature 4–6°C). A further 400 ml was infused retrogradely through a coronary sinus cannula. Flow through the coronary sinus was adjusted to maintain a pressure of < 60 mm Hg at the tip of the catheter. This resulted in flow rates of about 250 ml/min. Myocardial cooling was further enhanced by continuous pericardial irrigation with saline at 4°C. The aortic root was vented throughout the period of aortic cross clamping.

The distal graft anastomoses were then carried out, with sequential retrograde infusion of cardioplegic solution (200 ml) after completion of each graft or after 20 min, whichever occurred first. A "hot shot" of normothermic (37°C) cardioplegic solution was infused retrogradely after completion of the last distal anastomosis and before removal of the aortic cross clamp. Proximal anastomoses were carried out during systemic rewarming and the patient weaned off bypass at 37°C.

In group B, cardiopulmonary bypass was carried out at normothermia, keeping the nasopharyngeal temperature between 35 and 37°C throughout by active heating of the perfusate. The composition and method of delivery of warm blood cardioplegic solution have been described previously.⁹ In summary, oxygenated blood was taken from a side branch of the arterial line of the oxygenator, to which was added a variable concentration of potassium chloride via a syringe driver. No further additives were infused. Cardiac arrest was induced after aortic cross clamping by antero-

grade infusion of the cardioplegic solution into the aortic root, at a rate of 200 ml/min. Delivery was switched to a retrograde route when cardiac arrest had been achieved. Pressure at the tip of this cannula was measured continuously, and the flow adjusted to keep the pressure < 60 mm Hg. This resulted in flow rates of 200–250 ml/min, which were not interrupted throughout aortic clamping. Such a flow rate falls within the acceptable range.¹⁰

Potassium concentration of the cardioplegic solution was varied to ensure continued electromechanical arrest. The potassium concentration was 20 mmol/l during induction of arrest, but was reduced to 8–10 mmol/l for maintenance.

ASSAYS

Plasma oxidised glutathione (GSSG) was measured by the method of Abdalla *et al*¹¹ modified as described here. Fresh whole blood (1 ml) was added to a tube containing *N*-ethylmaleimide solution (1 ml). *N*-ethylmaleimide forms a complex with reduced glutathione (GSH) and prevents further formation of GSSG. The plasma was removed into a polypropylene tube after centrifugation at 2000g for 8 min and stored in liquid nitrogen until analysed. Thawed plasma (500 µl) was passed through a C18 solid phase extraction cartridge unendcapped (Technicol, Stockport, UK), which had been previously washed with methanol (1 ml) and deionised water (2 ml), and the cartridge rinsed with phosphate imidazole buffer (1 ml). The combined eluate was stored in chilled water and analysed without delay. A reagent blank (0.01 mol/l hydrochloric acid), an internal standard (250 nmol/l GSSG (Sigma, Poole, Dorset) in 0.01 mol/l hydrochloric acid, and a test plasma to which the equivalent of 250 nmol/l GSSG had been added (recovery plasma) were treated with *N*-ethylmaleimide and passed through the C18 cartridge as described above. Enzymatic analysis was carried out by mixing the eluate (700 µl) with a solution (300 µl) containing dithionitrobenzoic acid (0.87 mmol/l in a buffer of potassium phosphate (100 mmol/l), disodium EDTA (17.5 mmol/l) pH 7.5, and glutathione reductase (type IV; 5 units/ml) (Sigma) and 300 µl of NADPH (0.8 mmol/l) (Sigma). The reaction was monitored kinetically at 412 nm for 10 min. Plasma GSSG was calculated by reference to the blank corrected internal standard, after correcting for recovery indicated by the recovery plasma. A final correction for the blood haematocrit was made.

Plasma lactate was measured on a protein free sample obtained by mixing fresh whole blood (500 µl) with perchloric acid (0.6 mol/l) (1 ml). After mixing and centrifugation (2000 g for 10 min) the supernatant was stored in liquid nitrogen. The sample was thawed for analysis and measured using an enzymatic method utilising lactate dehydrogenase (Sigma). Serum creatine kinase MB isoenzyme was measured by an immuno-inhibition method (Sigma).

Figure 1 Cellular formation and removal of oxidised glutathione (GSSG) after free radical interaction with reduced glutathione (GSH).

Figure 2 Study protocol. Circles indicate sample collection.

Figure 3 Transmyocardial oxidised glutathione (GSSG) gradients in groups A and B. (Data are mean (SEM)). *** $P < 0.001$.

Figure 4 Transmyocardial lactate gradients in groups A and B. (Data are mean (SEM)). *** $P < 0.001$.

TRANSMYOCARDIAL OXIDATIVE STRESS AND ISCHAEMIA

Free radical interaction with GSH within the cell results in the increased formation and cellular release of GSSG (fig 1). This has been found to be a sensitive index of myocardial oxidative stress.¹²

Figure 2 shows the study protocol. Blood sampling for plasma GSSG and lactate, taken simultaneously from the coronary sinus and the aortic root, was performed just before application of the aortic cross clamp, and at 1, 5, 10, and 15 min after cross clamp release. Transmyocardial oxidative stress and ischaemia were assessed by the difference in plasma concentrations of GSSG and lactate respectively.

STATISTICAL ANALYSIS

Data were analysed by fitting a series of mathematical models by the method of maximum likelihood as described by Aitken¹³; this is equivalent to an analysis of variance, but permits the use of patients with incomplete data. Significant findings were evaluated in detail

using the Tukey multiple comparison test. Separate analyses were carried out on transmyocardial plasma GSSG, lactate, and serum creatine kinase MB isoenzyme differences. Best statistical estimates of the group means and their standard errors were obtained from the models fitted. Computations were performed using the GLIM 3.77 computer statistical package (NAG, Oxford). Although statistical significance was set at conventional levels, differences close to significance at the 5% level are also discussed.

Results

The table gives the baseline clinical characteristics of the study patients. Thirteen patients (mean (SEM) age 59.5 (2.6) years) were randomised to group A and 11 (mean (SEM) age 59.7 (3.3) years) to group B. There were no significant differences between the two groups. None of the study patients showed evidence of perioperative myocardial infarction on the basis of serial electrocardiographs or increased activity of serum creatine kinase MB isoenzyme (> 130 IU/l).

Figure 3 shows the transmyocardial GSSG gradients in groups A and B before and at timed intervals after cross clamp release. There was no significant change in transmyocardial stress after cross clamp removal in group B. In group A, however, there was a sharp increase within 1 min of cross clamp release, followed by a decline reaching baseline within 10 min. The changes over time were highly significant at 1 ($P < 0.001$) and 5 min ($P < 0.01$) relative to the pre-clamp baseline concentration. Significant differences between groups were apparent only at 1 and 5 min ($P < 0.001$) only.

Transmyocardial lactate gradients in group B showed no significant change after cross clamp removal (fig 4). In group A there was a sharp increase within 1 min of cross clamp release, followed by a decline reaching baseline within 10 min. The changes over time were highly significant at 1 ($P < 0.001$) and 5 min ($P < 0.01$) relative to the pre-clamp baseline concentration. Significant differences between the two groups were apparent at 1 min ($P < 0.001$). Significance was not reached at 5 min, although this was very close (significant at the 10% level).

Significant differences in the mean serum creatine kinase isoenzyme activity between the two groups were present at 2 ($P < 0.01$) but not 18 h after coronary artery bypass surgery (fig 5). A significant decrease was observed

Figure 5 Serum creatine kinase MB (CK MB) isoenzyme activity in groups A and B at 2 and 18 h after coronary artery bypass surgery (CABG). (Data are mean (SEM)). ** $P < 0.01$.

Patient characteristics

	Group A	Group B
No of patients	13	11
Sex ratio (M:F)	10:3	9:2
Mean (SEM) age (years)	59.5 (2.6)	59.7 (3.3)
Mean (SEM) bypass time (min)	122.2 (9.3)	127.5 (11.0)
Mean (SEM) cross clamp time (min)	63.1 (3.6)	66.3 (7.0)
Mean (SEM) conduits grafted	3.8 (0.2)	3.4 (0.3)

between 2 and 18 h in group A ($P < 0.01$); however, no significant change was present in group B.

Discussion

During open heart surgery, such as coronary artery bypass grafting, a period of aortic cross clamping and cardiac arrest is necessary to allow the procedure to be carried out. During this period, however, coronary arterial supply is interrupted resulting in global myocardial ischaemia. Reoxygenation when the cross clamp is released may result in oxidative stress and reperfusion injury. Rosenkranz and Buckberg¹⁴ have defined reperfusion injury as "those metabolic, functional and structural consequences of restoring coronary arterial flow . . . that can be avoided or reversed by modification of the conditions of reperfusion." This has been attributed to oxygen derived free radicals,¹⁵⁻¹⁷ which have been shown to be directly cytotoxic.¹⁸⁻²⁰ Clinically recognisable components of reperfusion injury include reperfusion arrhythmias, myocardial stunning, and lethal myocyte injury.²¹ Hence, functional recovery after bypass surgery may be impaired, and studies in humans have demonstrated that free radical sequestrants may reduce reperfusion injury after bypass surgery.²²⁻²⁵ Patients subjected to longer periods of global ischaemia induced during the cross clamp period showed a greater degree of oxidative stress during reperfusion and a significant delay in the rate of functional recovery than those with shorter cross clamp periods.¹²

Hypothermia and cardioplegic solutions are commonly used in an attempt to reduce cardiac metabolism and oxygen consumption, thereby minimising the ischaemic insult and hence oxidative stress after cross clamp release. In 1983, Rosenkranz and Buckberg¹⁴ showed that functional recovery could be significantly improved if the arresting and reperfusing solution was modified. They concluded that prevention of reperfusion damage was possible as the surgeon was in control of the temperature and composition of the reperfusate blood.¹⁴ A new concept of myocardial protection was introduced in 1989 using continuous warm blood cardioplegia and is being adopted in an increasing number of cardiac surgical centres.¹ Several reports have described its efficacy and safety in large series.²⁻⁷ Concerns that it may result in increased neurological damage (TD Martin *et al*, 29th annual meeting of the Society of Thoracic Surgeons, San Antonio, Texas, 1993) or inadequate right ventricular protection have not been substantiated (TD Martin *et al*, 29th annual meeting of the Society of Thoracic Surgeons).^{6, 26-29} It has been pointed out, however, that evidence of benefit in terms of myocardial metabolism and free radical activity is lacking.⁸ Therefore, this prospective randomised study has set out to directly examine differences between continuous, warm blood cardioplegia and the more standard use of intermittent hypothermic cardioplegia.

Previous coronary artery bypass surgery and surgical revascularisation studies have used indirect markers of free radical activity, such as plasma diene conjugates of 9,11-octa-decadieneoic acid and malonaldehyde.^{30, 31} However, their relation to free radicals and their accuracy have been subject to controversy.^{32, 33} Moreover, systemic heparin, which is routinely administered during bypass surgery, increases diene conjugate formation.³⁴ In light of the limitations and reservations imposed by the use of indirect biochemical markers we used GSSG, which reflects intracellular response to free radical generation and hence cellular oxidative stress.

Glutathione (GSH), a sulphhydryl containing tripeptide, is an abundant intracellular molecule that has a vital role in the cellular protection against oxygen derived free radical species. It acts as a co-substrate for glutathione peroxidase, reducing hydrogen peroxide and other peroxides to produce GSSG. Because the cell tries to maintain as high a GSH/GSSG ratio as possible, any increase in GSSG produces an active efflux of this molecule across the cell membrane to the plasma³⁵ (fig 1). Thus, the arteriovenous difference in plasma GSSG across the myocardium has been considered a sensitive indicator of myocardial oxidative stress.¹¹ In a similar manner, the arteriovenous difference in plasma lactate, formed from pyruvate under anoxic conditions,³⁶ may be considered an indicator of relative myocardial ischaemia.

Clear differences in transmyocardial ischaemia and oxidative stress between the two study groups were observed. A significant transmyocardial ischaemia was present for up to 5 min after cross clamp release in group A despite the use of hypothermia and cardioplegic solution. This was sufficient to cause a corresponding increase in transmyocardial oxidative stress over the same time period. In addition, evidence of increased myocyte damage, assessed by measurement of serum creatine kinase MB isoenzyme, was seen. It is unclear whether this damage is caused by ischaemia induced by cross clamping or subsequent oxidative stress. Either way, normothermic cardioplegia seems to avoid significant changes in ischaemic status and hence averts ensuing oxidative stress.

No patients showed electrocardiographic or serological evidence of perioperative infarction. Such patients would have been excluded from the analysis as the resulting increase in serum creatine kinase MB isoenzyme concentration would have distorted assessment of non-infarction myocardial damage.

This study provides direct evidence of enhanced myocardial protection by normothermic over cold cardioplegia. Global ischaemic reperfusion is a potential threat to viable myocardium that may impair functional recovery after bypass surgery. Continuous normothermic cardioplegia is technically more difficult, however, because of increased blood around the operative field.⁶ It remains to be seen whether all patients would benefit significantly from this technique.

Patients with established poor ventricular function or those who have already suffered a prolonged ischaemic insult (such as after acute infarction or abrupt coronary artery occlusion from interventional angioplasty) and who may not be able to tolerate further iatrogenic ischaemic myocardial injury may have more to gain from normothermic cardioplegia than those with well preserved ventricular function.

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