Blood cardioplegia filtration

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The introduction of blood cardioplegia has been proven to limit ischaemia and reperfusion injury in cardiac surgery. But the presence of activated neutrophils in the capillary bed may cause further damage. Leukocyte filters have been shown to be very effective in reducing the leukocytes in blood cardioplegia to less than 10%. Leukocyte depletion of blood cardioplegia provides an excellent approach to minimizing myocardial injury, predominantly in high-risk cardiac surgery. Perfusion (2003) 18, 75–80.

Introduction

Restoration of blood flow after myocardial ischaemia is accompanied by a variety of cytotoxic effects. This may result in additional myocardial damage and unfavourable operative outcome, especially in patients with ischaemically compromised myocardium or severely impaired ejection fraction.

Several experimental and clinical studies have proven the role of neutrophils in the pathophysiology of myocardial ischaemia and reperfusion. Ischaemia itself induces an immediate polymorphonuclear leukocyte response and activates granulocytes via the complement cascade. Ischaemic endothelial cells express membrane receptors for immunoglobulin, complement fragments and adhesion molecules, which promote adhesion, plugging and migration of neutrophils. In addition, the release of nitric oxide and adenosine by the endothelial cells is markedly reduced after ischemia. Nitric oxide and adenosine exert a cardioprotective effect via the inhibition of neutrophil activities. Complement components and other metabolites of ischaemic myocardium cause large polymorphonuclear leukocytes to become stiffer and less capable of smoothly traversing capillaries. As a consequence, neutrophil granulocytes may plug capillaries during ischaemia and this may result in incomplete restoration of blood flow after the start of reperfusion. Production of additional chemoattractants by activated neutrophils amplifies the initial inflammatory response. Inhibition of neutrophil adherence by blocking neutrophil adhesion molecules during reperfusion after heart transplantation surgery resulted in prevention of myocardial stunning, contracture, low reflow and oedema.

Another very important factor is the activation of neutrophils, complement cascade and other inflammatory mediators initiated during cardiopulmonary bypass. Neutrophil granulocytes may cause myocardial damage due to the production of large quantities of oxygen-derived free radicals (respiratory burst), i.e., superoxide anion, hydrogen peroxide, hydroxyl radical, hypochlorous acid and chloramine.

Reperfusion damage after myocardial ischaemia can be avoided or significantly ameliorated by applying a controlled reperfusion with warm substrate-enriched blood cardioplegia before aortic crossclamping is released. Warm blood cardioplegic solutions are, therefore, often used to limit the reperfusion injury following cardioplegic arrest in cardiac surgery. Blood cardioplegia has been proven to be superior to crystalloid solutions with regard to oxygen supply, prevention of myocardial oedema, antioxidative capacity and replenishment of substrates for myocardial metabolism. However, the presence of leukocytes and platelets in the capillary bed can cause further damage. Despite the good results with warm cardioplegic infusions during the initial phase of reperfusion, recovery of the myocardium may be incomplete if the heart is subjected to a more severe stress (e.g., long ischaemic time in cardiac transplantation, unstable angina, evolving myocardial infarction). After severe ischaemia, the myocardium is very susceptible to the harmful effects of neutrophils. In this situation, controlled reperfusion with blood cardioplegia during aortic crossclamping provides excellent access to the application of leukocyte filters. Blood cardi-
Ooplegia in adult patients is applied at a flow rate of 150–300 mL/min. In contrast to the arterial line leukoreducing filters at flow rates of about 5 L/min, the blood cardioplegia leukofilter displays predictably different leukoreductive properties since it was designed for filtration at 500 mL/min. Using modern leukocyte filters, it is possible to remove more than 90% of the leukocytes from the blood cardioplegic solution up to a total volume of 1300 mL (Figure 1).

**Experimental results**

**In vitro studies.** In an isolated rat model, hearts were arrested for 60 minutes, with warm blood cardioplegia given at 20-minute intervals. The use of a leukocyte filter (Sepacell PLX; Asahi Medical Co., Ltd, Tokyo, Japan) resulted in better preserved endothelial function and left ventricular diastolic compliance. In neonatal lambs, reperfusion with leukocyte-depleted blood (Sepacell) after two hours of cardioplegic arrest was followed by increased left ventricular developed pressure and improved endothelial response to acetylcholine.

**Experimental models of myocardial infarction.** Kofsky et al. tested the effect of leukocyte depletion during reperfusion in a dog model after two hours of regional myocardial ischaemia. Compared to early reperfusion with unmodified blood, leukocyte depletion resulted in a lower incidence of ventricular fibrillation, decreased coronary vascular resistance and limited histochemical damage. In contrast, improvement of regional contractility could not be observed after leukocyte filtration, but only when blood cardioplegia was used. Neutrophil filtration of blood cardioplegic solutions did not further enhance the salutary effects of blood cardioplegia.

Similar results were obtained by Byrne et al. after 90 minutes of regional myocardial ischaemia. Leukocyte filtration versus reperfusion with unmodified whole blood improved the left ventricular stroke work index, mean rise in left ventricular pressure and myocardial blood flow. No other adjuncts to controlled reperfusion, e.g., substrate-enriched blood cardioplegia, were given in this study.

Schmidt et al. investigated leukocyte-depleted blood cardioplegia in a canine model of 90 minutes of regional myocardial ischaemia, followed by a 60-minute cardioprotective arrest using continuous blood cardioplegia. They found no effect on global ventricular function or water content, but the endothelial function was significantly better preserved in the leukocyte-depleted group.

Bolling et al. used the Pall BC-1 leukocyte-depleting filter in a neonatal piglet model to achieve a systemic leukocyte depletion at flow rates of 350–500 mL/min. They could demonstrate a significant attenuation of myocardial injury due to abrupt reoxygenation of hypoxic myocardium. Systemic leukocyte count fell abruptly with the initiation of bypass to $1.9 \pm 0.4 \times 10^3$ cells/mm$^3$ and remained low during the entire 90 minutes of CPB.

**Experimental heart transplantation.** In an isolated pig heart model, Breda et al. could show that reperfusion of the myocardium after 12 hours of hypothermic cardioplegic storage with neutrophil-depleted blood was associated with improved post-ischaemic function, a threefold higher coronary flow, and preserved ultrastructure of the myocardium compared to the control group.

Yamamoto et al. used the Pall BC1B for systemic leukocyte depletion in a dog model of orthotopic heart transplantation and could remove about 80% of the white blood cells during the initial reperfusion. Leukocyte depletion resulted in improved contractility and better preserved endothelial function compared to the control group.

Fukushima et al. compared leukocyte-depleted blood cardioplegia versus undepleted blood cardioplegia after 24-hour preservation of canine hearts for heterotopic transplantation. Only leukocyte-depleted blood cardioplegia was able to replenish the energy-depleted myocardium, preserve coronary flow and contractile function, and to reduce lipid peroxidation.

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**Figure 1** Leukocyte depletion of porcine blood using the Pall BC1B. Note: the capacity of the filter has been exhausted after a total blood volume of approximately 1300 mL. To maintain a leukocyte depletion of more than 90%, the filter has to be replaced.
Gundry et al.\textsuperscript{20} used leukocyte-depleted blood cardioplegia for the initial reperfusion after orthotopic heart transplantation from non-heart-beating donors in a juvenile baboon model.

We have described a technique for successful orthotopic transplantation of pig hearts from non-heart-beating donors after 30 minutes of normothermic ischaemia without donor pretreatment.\textsuperscript{21,22} In this experimental model, reperfusion with unfiltered blood cardioplegia after 30 minutes of unprotected normothermic ischaemia resulted in ischaemic contracture (‘stone heart’) within a few minutes after the start of reperfusion. It was not possible to wean the hearts from extracorporeal circulation despite controlled reperfusion with substrate-enriched blood cardioplegia. After addition of a leukocyte blood cardioplegia filter to the reperfusion regime, cardiac contractility was markedly improved and the animals could be weaned successfully. Haemodynamic measurements 24 hours after heart transplantation revealed no significant difference between the non-heart-beating donors and a control group transplanted from beating heart donors. Post mortem examination showed only minimal histological damage of the myocardium.\textsuperscript{23}

**Clinical studies (Table 1)**

**Routine CABC.** In patients undergoing elective open-heart surgery, the use of a leukocyte-depleting filter for blood cardioplegia resulted in a significantly diminished release of creatinine kinase and troponin T as compared to the control group.\textsuperscript{24} A study on 160 routine CABC patients demonstrated a significant improvement in myocardial protection. This was shown by an improved cardiac index, diminished incidence of ventricular fibrillation, and lower dosage of antiarrhythmics in the filter group.\textsuperscript{25} Moreover, the authors found a significant decrease in myocardial enzyme release, suggesting diminished myocardial cell injury.

Using cold blood cardioplegia, Ichihara et al.\textsuperscript{26} have shown lower values of lipid peroxide, elastase and CK-MB in the leukocyte depletion group. Hayashi et al. found significantly reduced myocardial cell injury due to the use of leukocyte-depleted blood cardioplegia (e.g., lower malondialdehyde levels in the coronary sinus blood).\textsuperscript{27} In contrast, Browning et al. could not find any benefit of leukocyte filtration in routine coronary bypass patients despite the leukocyte count being reduced by a mean of 90.7%.\textsuperscript{28} No significant differences were found compared to the control group for postreperfusion oxidized glutathione gradients, CK-MB, troponin T or in the frequency of postoperative complications.

**Emergency cardiac surgery or depressed ventricular function.** In a randomized study, De Vecchi et al. investigated the effect of leukocyte-depleted blood cardioplegia in patients with normal and depressed ventricular function undergoing coronary bypass.\textsuperscript{29} In patients with depressed ejection fraction, the recovery rate of plasma glutathione redox ratio (oxidized/total glutathione) was significantly faster in the leukocyte-depleted versus con-

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**Table 1 Clinical studies with leukocyte-filtred blood cardioplegia**

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**Blood cardioplegia filtration**

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control group. In patients with a normal ejection fraction, leukocyte depletion had no beneficial effect.

Sawa et al. tested the effect of leukocyte filtration of blood cardioplegia for initial reperfusion after coronary bypass grafting in elective versus emergency surgery. They found significantly lower peak creatinine kinase-MB levels, lower myocardial malondialdehyde release, and lower inotropic support when leukocyte-depleted blood cardioplegia was used. These observations could be made only in emergency surgical procedures requiring a preoperative intra-aortic balloon pump because of developing acute myocardial infarction. In elective coronary artery bypass grafting, no benefit of leukocyte filtration was found due to the clinical data. In another study, the same authors evaluated the effect of leukocyte-depleted terminal blood cardioplegia in patients with left ventricular hypertrophy undergoing aortic valve replacement. Biopsies were taken 15 minutes after the start of reperfusion. Leukocyte depletion resulted in diminished myocardial and endothelial damage, fewer neutrophil adhering to endothelial cells, lower malondialdehyde and creatinine kinase release and lower requirement for inotropic support.

To assess the effect of leukocyte filtration on oxidative stress, Pala et al. measured total oxidized (GSSX) and reduced glutathione (GSH) in coronary sinus plasma of elective coronary bypass patients. They demonstrated a benefit of leukocyte depletion in patients with an ejection fraction of less than 35% compared to patients with normal left ventricular function.

In a cohort of 32 patients with coronary artery disease and an ejection fraction of less than 35% undergoing bypass surgery, leukocyte filtration of blood cardioplegia resulted in lower myocardial release of troponin T, higher postoperative ejection fraction and lower catecholamine demand. These results are consistent with the experience that the specific contribution of neutrophils to mild or moderate myocardial damage seems less effective in contrast to the severely damaged myocardium.

**Cardiac transplantation.** Inadequate myocardial protection associated with ischemic and reperfusion injury is an important reason for early graft failure after heart transplantation. Moreover, suboptimal early graft function is common, and high-dose inotropic support is frequently required postoperatively. Due to the increased risk of early graft failure, a total ischemic time beyond four hours is avoided by most transplant physicians.

Reperfusion with substrate-enriched, leukocyte-depleted blood cardioplogic solution for the first three minutes of reperfusion and leukocyte-depleted blood for additional seven minutes after prolonged hypothermic ischemia resulted in shorter duration of inotropic support, decreased leakage of myocardial enzymes and prevented ultrastructural injury.

**Conclusions**

1) Controlled reperfusion with blood cardioplegia during aortic crossclamping provides excellent access to the application of leukocyte filters.

2) The most benefit of leukocyte depletion can be expected in ischaemically compromised hearts (e.g., long aortic crossclamping time, long conservation time in heart transplantation, evolving myocardial infarction) and in hearts with impaired ventricular function.

3) Leukocyte depletion is most effective during early reperfusion.

4) The leukocyte filter should extract about 90% of the leukocytes from the initial reperfusate to provide a maximal beneficial effect.

**References**


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