Myocardial Protection With Leukocyte Depletion in Cardiac Surgery

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A role of neutrophils in ischemia-reperfusion injury has been focused on as one of the mediating factors of inflammatory reactions. Current studies have reported the efficacy of leukocyte-depletion in reperfusion by using leukocyte removal filter to attenuate reperfusion injury during open heart surgeries. For clinical application, we have introduced leukocyte-depleted terminal blood cardioplegia (LDTC) in adult patients and leukocyte-depleted blood cardioplegia in pediatric patients. The results of elective surgery in noncompromised LDTC did not significantly alter the results in terms of leakage of creatine kinase (CK)-MB, production of malondialdehyde from myocardium, and dopamine dose required at the weaning from cardiopulmonary bypass compared with the whole-blood reperfusion or with terminal cardioplegia alone. In contrast, the results in emergency coronary artery bypass graft (CABG) patients differed significantly between the LDTC group and the other two groups. Leukocyte-depleted reperfusion was also effective in a similar fashion for patients with severe left ventricular hypertrophy caused by chronic aortic valve disease. Leukocyte-depleted blood cardioplegia was useful in pediatric patients. Thus, leukocyte depletion may be beneficial as an adjunct to terminal blood cardioplegia or blood cardioplegia during cardiac surgery to attenuate leukocyte-mediated ischemia-reperfusion injury in patients with compromised hearts, such as those with preoperative ischemic insults, severe left ventricular hypertrophy, and in pediatric patients.

Key words: Blood cardioplegia, terminal cardioplegia, leukocyte depletion, ischemia reperfusion injury, hypertrophy.

Recently, several evidences have indicated that activated neutrophils contribute to myocardial reperfusion injury after ischemia. Histopathologically, neutrophils have been shown to mechanically obstruct capillaries, thereby inhibiting reperfusion of ischemic tissue, known as the *no-reflow phenomenon.*[1,2] The generation of oxygen-derived free radicals, the release of proteolytic enzymes by filtrating neutrophils, or both processes also cause histologic and functional derangement of myocardial tissue.[3] These findings suggest the possible therapeutic effect of leukocyte depletion by using a leukocyte removal filter, which has been found to reduce the extent of myocardial damage after reperfusion.[4-9]

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In this study, we reviewed the role of neutrophils in myocardial reperfusion injury, and then introduced the method of leukocyte depletion adjunct to terminal blood cardioplegia,[9,10] particularly in patients with compromised hearts, or to blood cardioplegia, especially for pediatric patients to attenuate leukocyte-mediated ischemia reperfusion injury.

Pathophysiology of Ischemia Reperfusion Injury In Myocardium

Although myocardial protection during cardiac surgery showed great advances, myocardial damage caused by ischemia reperfusion is still one of the important problems that needs to be solved to improve clinical results.[11-15] Because of a high-energy metabolism, discontinuation of blood supply to the myocardium is likely to disturb tissue perfusion and to cause the progression of myocardial injury in to irreversible damage within a few minutes. However, reperfusion is indispensable in the recovery of myocardial injury during ischemia, it can aggravate myocardial injury depending on the length of ischemic period.

Although reoxygenation of tissue by reperfusion is theoretically expected to improve ischemia, myocardial damage caused by ischemia is exacerbated after reperfusion. This fact has suggested a possibility that oxygen radicals are responsible for reperfusion injury in myocardium. Numerous investigators have shown that oxygen-derived free radicals are closely involved in reperfusion injury in myocardium. It is known that various scavengers for free radicals are present in organisms for host defence. It seems likely that reperfusion injury is caused by oxygen radicals when the production of oxygen radicals after reperfusion is greater than the defensive systems, which is disturbed by ischemia. Oxygen radicals are known to peroxidate phospholipids and other cell components, causing degeneration of these cells. Lipid peroxydation caused by oxygen radicals leads to the degeneration of membranous organelles such as sarcolemma, mitochondria, and sarcoplasmic reticulum. The resultant increase of calcium permeability across membranes and the decrease in mitochondrial cell damage causes myocardial necrosis. Hearse et al reported an "oxygen paradox," showing the progression of myocardial injury at the time of reoxygenation after hypoxia.[14] They suggested that oxygen radicals and calcium influx are involved in this phenomenon.

Regarding the mechanism of oxygen radical production after reperfusion, it is well known that xanthine, which is the final product of ischemia-induced adenosine triphosphate (ATP) break-down, serves as a substrate for xanthine oxidase and stimulates the production of oxygen radicals. In human hearts, however, xanthine oxidase activity is very low, and it seems unlikely that oxygen radical production after reperfusion can be explained by this pathway alone.[15] It seems possible that ischemia causes uncontrolled leakage of electrons from the electron transmission system of intracellular organelles such as mitochondria, and that these electrons reduce oxygen, resulting in the production of superoxide anion. Neutrophils are also attracting much attention as a possible major source of oxygen radicals,[1] and will be described in detail in other articles in this issue.

Role of Neutrophils In Myocardial Reperfusion Injury

The role of leukocytes in myocardial reperfusion injury has been reported, and the main cause of its worsening effects is the capillary plugging by leukocytes after myocardial ischemia and reperfusion in dogs as reported by Engler et al.[4] They were also the first to determine the efficacy of leukocyte depletion on the no-reflow phenomenon in canine hearts subjected to ischemia and reperfusion.[4] Additional early evidence of leukocyte involvement in myocardial reperfusion injury was the administration of neutrophil antiserum to dogs.[16] Several subsequent studies have reported the efficacy of leukocyte removal filters in attenuation of reperfusion injury.[17] Neutropenia induced by leukocyte removal filters reduced microvascular permeability, suggesting that neutrophils are largely responsible for coronary endothelial dysfunction.[18] It is, therefore, doubtless that neutrophils play a significant role in myocardial reperfusion injury (Fig 1).

Activation of neutrophils can be easily evoked by the complement activation during open heart surgery.[19] When activated, neutrophils produce superoxide anions by means of the reduced form

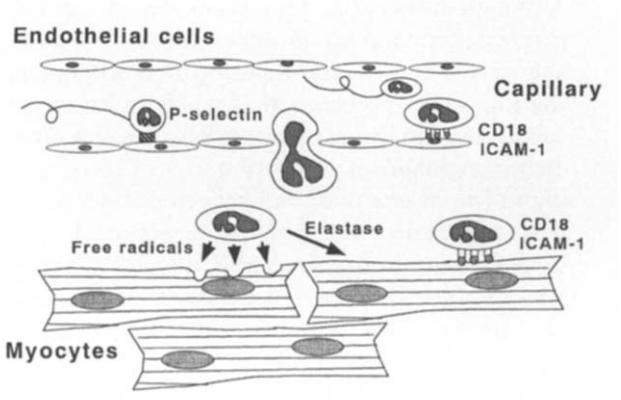

Figure 1. Scheme of neutrophil-mediated reperfusion injury.

of nicotinamide-adenine di-nucleotide phosphate (NADPH) oxidase. Furthermore, they release myeloperoxidase, which catalyzes the production of OCl−, a greater oxygen radical. Neutrophils also produce NO, which can react with superoxide anions to yield ONOO−, one of the most active radicals.[20] Therefore, activation of neutrophils produces various oxygen radicals exacerbating reperfusion injury.

Neutrophil infiltration in reperfused myocardium is an initial and critical step.[21] Once the neutrophil adheres to the vascular endothelial cells, transendothelial migration, extravascular migration to neighboring cardiac myocytes, and, finally, cardiac myocyte injury can occur.[21] For the adhesion of neutrophils to coronary endothelial cells and myocytes, the expression of adhesion molecules is essential. Recently, several adhesion molecules such as P-selectin, intercellular adhesion molecule-1 (ICAM-1), platelet endothelial cellular adhesion molecule-1 (PECAM-1), and so forth, have been shown to be involved in this mechanism.[21] It has been reported that P-selectin on endothelial cells plays an important role in the rolling phenomenon,[22] an initial process of neutrophil adhesion to endothelial cells. ICAM-1 on endothelial cells are thought to confirm the adhesion of neutrophils to endothelial cells and myocytes.[23] PECAM-1 may play a role in the migration of neutrophils through endothelial cells.

However, the factors that promote neutrophil migration to damaged areas caused by ischemia and reperfusion remains uncertain.[21] We have performed an in vitro experiment in which a coculture system with myocytes and coronary endothelial cells was prepared by using the Boyden chamber method to realize the neutrophil migration through endothelial cells.[24] This experiment revealed that hypoxic myocytes produce chemotactic factors that can serve as a signaling factor for neutrophils to migrate into the damaged area in myocardium.

Leukocyte-Depleted Terminal Cardioplegia

Although several experimental findings have shown the efficacy of leukocyte removal filters in attenuation of reperfusion injury, no attempt has been made to apply this technique to the patients except by Pearl et al[25,26] who reported that leukocyte-depleted reperfusion improved the graft function in the transplanted human hearts. We were the first to apply the leukocyte-depleted reperfusion as an adjunct to terminal cardioplegia, the so-called *leukocyte-depleted terminal blood cardioplegia* (LDTC), in patients during open heart surgery.[9,10] The method of LDTC is described in detail later.

Method of Leukocyte-Depleted Terminal Cardioplegia

After completion of the surgical procedure during cross-clamping of the aorta, arterial blood for LDTC was separately circulated from the oxygenated reservoir (Fig 2). A newly developed leukocyte removal filter (Cellsorba-80P; Asahi Medical Co, Tokyo, Japan) for coronary perfusion with heparinized blood was incorporated just after the oxygenator reservoir to deplete leukocytes. This filter consists of nonwoven fine polyester fiber wound around a porous cylinder. The blood is passed through the polyester fiber at a flow rate of 30 mL/min for 10 minutes. Then, arterial blood was mixed with cold potassium crystalloid cardioplegic solution by a newly developed computerized system with double-head coupled roller pumps (Senko Ika Co, Tokyo, Japan). It was infused into the aorta antegrade or into the coronary sinus retrograde through a cardioplegic cannula at a flow rate of 1 mL/min/g of left ventricule (LV) mass with a perfusion pressure of no higher than 60 mm Hg for 10 minutes. It was started at 30°C and was heated immediately to 36°C. The hematocrit was maintained at 15% to 20%, and the level of potassium was maintained from 8 to 10 mEq/L by controlling the ratio of arterial blood and potassium crystalloid cardioplegia with a computerized system of double-head coupled roller pumps (Table 1). At the end of LDTC reperfusion, the aorta was unclamped and the heart was reperfused with oxygenated whole blood through the aortic cannula.

Results In Patients With Coronary Artery Bypass Grafting

We applied LDTC in a selected group of 68 patients with coronary artery bypass grafting (CABG) either for elective surgical procedures (n = 38) or emergency surgical procedures (n = 30) with the use of a preoperative intra-aortic balloon pump (IABP) because of developing

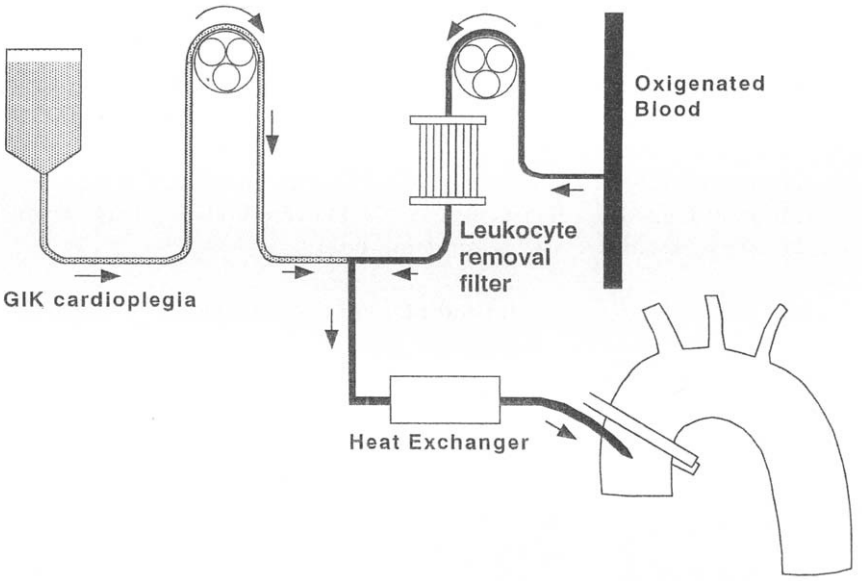

Figure 2. Scheme of a computerized system for leukocyte-depleted terminal cardioplegia.

acute myocardial infarction. Patients were randomized into 3 groups for reperfusion: whole-blood (WB) group, terminal cardioplegic solution (TC) group, and LDTC group. The clinical results of elective surgery, which can cause relatively mild reperfusion injury the same as stunned myocardium, in the LDTC group (n = 13) did not differ significantly from those in the WB (n = 15) and TC groups (n = 10). However, in emergency CABG, the LDTC group (n = 10) showed significantly lower peak creatine kinase (CK) MB levels and maximum dopamine dose required at the weaning of cardiopulmonary bypass than did the WB (n = 10) and TC groups (n = 10) (Fig 3). Moreover, the LDTC group showed a significantly lower difference of malondialdehyde between arterial and coronary sinus blood than the TC and WB groups.

Table 1. Protocol for Leukocyte-Depleted Terminal Cardioplegia

Blood vs crystalloid cardioplegia	2:1
Potassium	16 mEq/L
Sodium	130 mEq/L
Calcium	1.0 mEq/L
Osmolarity	280 mOsm/L
Perfusion pressure	40-60 mm Hg
Perfusion flow	1 mL/min/gm
Hematocrit	15%–20%
Perfusion temperature	30°C–36°C

Results In Patients With LV Hypertrophy

Furthermore, leukocyte-depleted reperfusion was also effective in cases complicated by LV hypertrophy (LV mass > 300 g, left ventricular end systemic volume index [LVESVI] > 100 mL/m2).[10] We applied this method in a selected group of 30 patients undergoing aortic valve replacement. Patients were randomized into 3 groups for reperfusion: WB group, (n = 10), TC (n = 10), and LDTC (n = 10). LV biopsies for ultrastructural assessment were obtained before and after ischemia and 10 minutes after reperfusion. Semiquantitative scoring for ultrastructural alterations indicated that the LDTC group achieved significantly better recoveries of both scores at reperfusion for mitochondrial damage of myocytes and for endothelial cell damage of capillaries than the TC and WB group in which plugging neutrophil in the capillary was detected morphologically (Fig 4). The LDTC group showed significantly fewer neutrophils adhering to endothelial cells at reperfusion, and a lower value of malondialdehyde derived from myocardium than the WB and TC groups. Regarding the clinical data, the LDTC group showed a lower maximum CK-MB and a higher percentage of spontaneous defibrillation and a lower requirement for dopamine than did the WB group, whereas the TC group failed to improve in these clinical data.

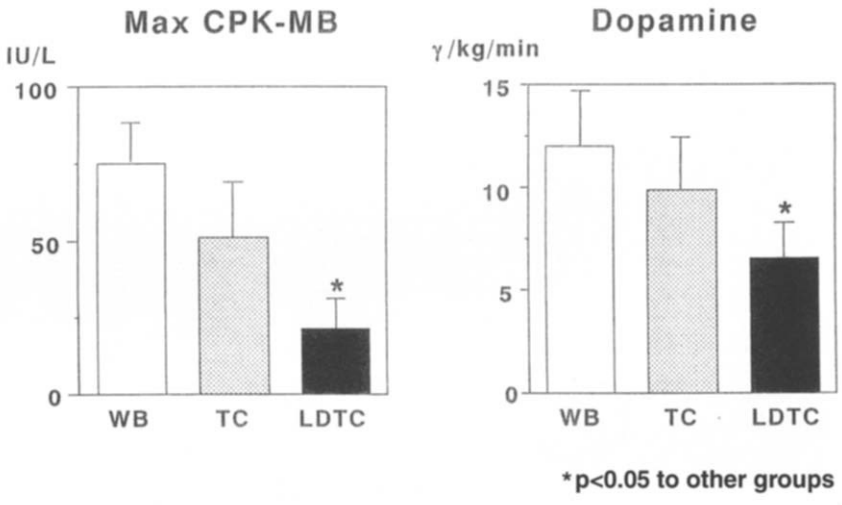

Figure 3. Comparison of CK-MB and dopamine dose at the time of weaning from cardiopulmonary bypass among WB, TC, and LDTC cases in patients with emergency CABG.

Leukocyte-Depleted Blood Cardioplegia

Fifty pediatric patients who underwent open heart surgery for congenital heart disease between January 1997 and March 1999 were reviewed.[27] Twenty-five were administered leukocyte depleted blood cardioplegia (LDBCP) for myocardial protection during ischemic periods (LDBCP group) and the remaining 25 were given blood cardioplegia (BCP) without leukocyte depletion (BCP group) (Fig 5). The difference in plasma concentrations of malondialdehyde between coronary sinus effluent and arterial blood just after reperfusion in the LDBCP group was significantly lower than that in the BCP group. The LDBCP group showed significantly lower plasma concentrations of human heart fatty acid–binding protein at 50 minutes after reperfusion and the peak value of CK-MB during the first 24 postoperative hours than did the BCP group. The maximum dose of catecholamine was significantly smaller in the LDBCP group (Fig 6). In the LDBCP group, the difference of malonedialdehyde (MDA) between coronary sinus effluent and arterial blood concentrations just after reperfusion, the human heart fatty acid–binding protein (HH-FABP) concentration obtained at 50 minutes after aortic unclamping, the peak value of CK-MB for the first 24 hours postoperatively, and the maximum dose of catecholamine at the time of weaning from cardiopulmonary bypass (CPB) and during postoperative course were lower than corresponding values in the BCP

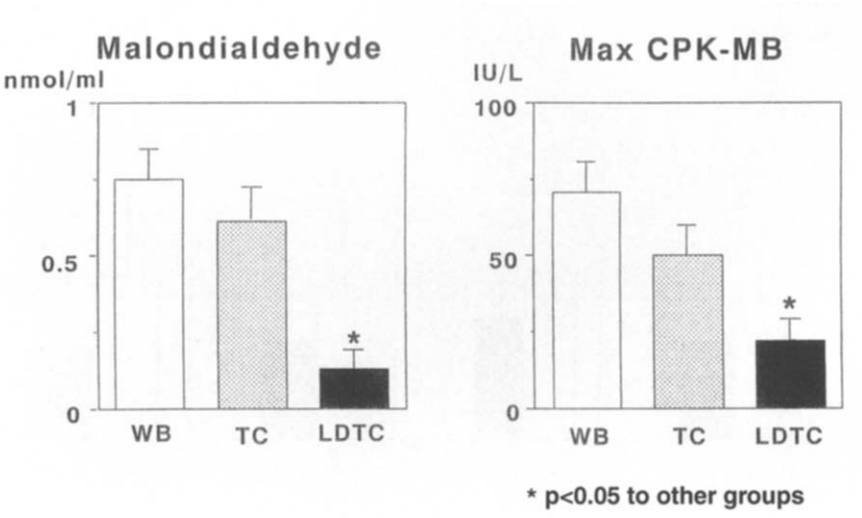

Figure 4. Comparison of malondialdehyde in myocardium and CK-MB among WB, TC, and LDTC cases in patients with LV hypertrophy.

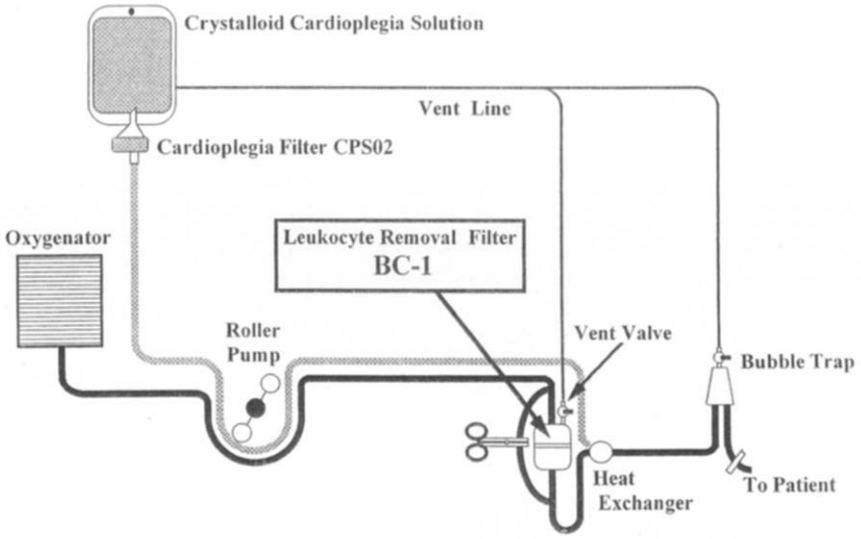

Figure 5. Scheme of a system for leukocyte-depleted blood cardioplegia.

group. A quantitative functional-morphologic evaluation was not performed in this study because of the study design, but LDBCP seems to be effective in pediatric patients and such experimental and clinical evaluations would be of interest for future study.

Technical Consideration of Cardioplegic Leukocyte Filtration

The principle of LDTC or LDBCP seem to be practically useful because of the technical convenience and the improvement of the existing methods of myocardial protection. Terminal cardioplegia has been reported to accelerate myocardial metabolic recovery and preserve high-energy phosphate.[28-30] This may contribute to attenuation of the damage mainly in myocytes. On the other hand, leukocyte-depleted reperfusion alone may have other contributions to attenuate the damages in the endothelial cells, mainly caused by neutrophil adherence to endothelial cells. Therefore, the combination of both terminal cardioplegia and leukocyte-depleted reperfusion in LDTC seems to provide a better preservation of the myocardium than terminal cardioplegia alone or leukocyte-depleted reperfusion alone.[9]

With respect to the clinical application of reperfusion with leukocyte-depleted blood, the direct insertion of the leukocyte removal filter into the circuit of the extracorporeal circulation is easy compared with our method.[27] In that case, however, it remains to be determined whether completeness of leukocyte depletion in the systemic circulation would lead to clinical prob-

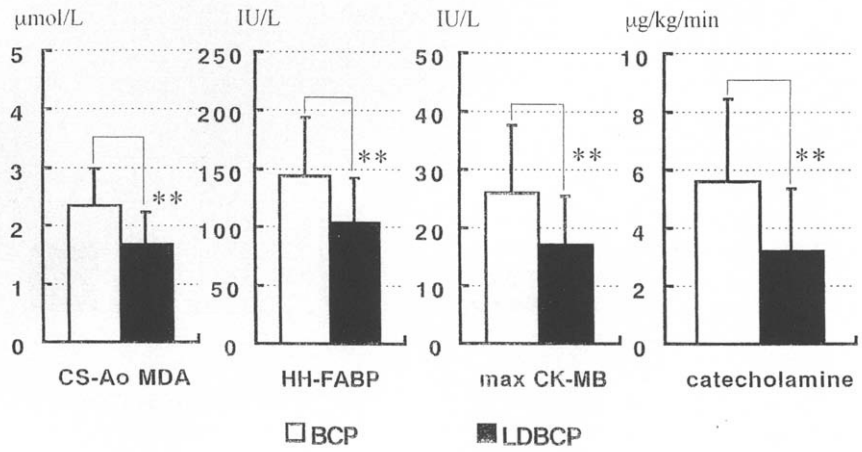

Figure 6. Comparison of MDA, hhFABP, max CPK-MB, and catecholamine between BCP and LDBCP groups. □, BCP; ■, LDBCP. $^{**}P < 0.05$.

lems.[9] Conversely, incomplete depletion of leukocytes provided no other attenuation in the clinical outcome. In our LDTC reperfusion system, terminal cardioplegia was applied for selective perfusion with leukocyte-depleted blood only through the coronary circulation and only during the early phase of reperfusion. Consequently, no serious complications occurred in patients despite more than 95% depletion of leukocytes in the coronary circulation.

It is still unclear whether BCP solution containing leukocytes and platelets has a cytotoxic effect on the myocardium during periods of ischemia. CPB-induced blood activation[31] may enhance capillary plugging and release cytotoxic chemical mediators before cardioplegic cardiac arrest and during aortic cross-clamping. Nakanishi et al[32] showed that 45 minutes of normothermic ischemia plus intermittent BCP without reperfusion resulted in no significant coronary endothelial dysfunction related to nitric oxide in adult mongrel dogs. Schmidt et al[33] reported a protective effect on endothelial function by leukocyte depletion in both extracorporeal circuits and cardioplegia circuits by using continuous BCP under moderate hypothermia in a canine model of regional myocardial ischemia and reperfusion. However, the possible cytotoxicity of BCP as a risk for myocardial reperfusion injury has not been clearly shown, even under conditions of cold BCP administered to hypothermic myocardium during cardioplegic arrest. Our results suggest the superiority of LDBCP for myocardial protection during open heart surgery with respect to MDA, HH-FABP, maximum CK-MB, and catecholamine dosage, even though the study group comprised infants, in whom the myocardial scavenging systems for CPB-induced chemotactic mediators are immature in comparison with adult myocardium.[2,34,35] However, two disadvantages of the LDBCP system prevent us from using it unquestioningly. In pediatric open heart surgical procedures, the dose of BCP is smaller than that in adult surgeries, and it seems possible to frequently repeat the leukocyte depletion. In cases requiring many administrations of BCP, however, repeated LDBCP administration lessens the ability of the filter to deplete the leukocytes; it is affected by the total BCP administered. Because the mechanism of possible cytotoxicity of BCP remains unclear, we are not willing to make the best use of LDBCP in such cases, and our first choice is to use leukocyte-depleted terminal cardioplegia solution, which we use in adult open heart surgeries.[9,10] Another disadvantage is the priming volume required for the LDBCP system. The leukocyte removal filter requires as much as 200 mL for priming, and this amount is large in comparison with the total volume used for priming the pediatric CPB circuit. The increased priming volume for CPB circuits enhances hemodilution and may prevent cardiac surgeries without blood components, especially in pediatric patients. Although frequent leukocyte depletion can be used in pediatric open heart surgeries, we have to use conventional BCP administration without leukocyte depletion in cases in which perioperative blood transfusion should be avoided. Our hope is that an improved system will be developed that will allow for frequent leukocyte depletion along with a smaller priming volume.

Future Perspectives

It is still controversial whether neutrophils are involved in stunned myocardium.[36] In our study, the results of LDTC in elective CABG surgery, which can cause relatively mild reperfusion injury the same as stunned myocardium, did not differ significantly from those in the WB reperfusion group or the group with terminal cardioplegia alone. This result is consistent with the past reports on the basis of an animal model that denied their involvement in the mechanisms of stunned myocardium.[36] It is reasonable that the role of neutrophils in reperfusion injury may depend on the degree of myocardial damage, suggesting the limiting effect of leukocyte depletion in the stunned myocardium. However, the results of both emergency CABG and aortic valve replacement (AVR) with LV hypertrophy, which can cause severe reperfusion injury, differed significantly between the LDTC group and any of the other two groups. These results suggest that the removal of leukocytes is useful when serious reperfusion injury is expected to occur during open heart surgery, especially in compromised hearts, including preoperative myocardial infarction[9] or the hypertrophied heart,[10] and that its role will be greater when preceeding ischemic damage is more severe.

Regarding the duration of reperfusion with leukocyte-depleted blood, our application of leu-

kocyte-depleted reperfusion as an adjunct to terminal cardioplegia consisted of the controlled reperfusion only for the coronary circulation with leukocyte-depleted blood during 10 minutes of reperfusion. These first 10 minutes of reperfusion are the most critical period for the morphologic changes of subcellular levels such as neutrophil plugging in consequence with the no-reflow phenomenon.[14,15] We performed the LV biopsy and evaluated the subcellular alteration for the comparison of the myocardial protective effects.[10,37] Consequently, this 10 minutes of LDTC appears to be enough for patients, even those with LV hypertrophy, to attenuate the neutrophil-mediated reperfusion injury, though the hearts reperfused with 10 minutes of LDTC were nullified by the subsequent perfusion with WB.

As for the pathogenesis of reperfusion injury in myocardium, a number of factors can be proposed to explain it.[14,15] Therefore, to improve the myocardial protection, particularly in patients with compromised hearts, the combination of myocardial protective methods from the standpoints of several mechanisms might be required, and LDTC appears to be one of the good candidates for this strategy. Further studies seem to be essential for us to advance myocardial protection as one of the future perspectives.

Summary

Leukocyte depletion may be beneficial at the time of reperfusion, particularly as an adjunct to terminal cardioplegia or blood cardioplegia during cardiac surgery to attenuate reperfusion injury, especially in adult patients with a compromised heart or in pediatric patients. Removal of leukocytes appears to be useful when serious reperfusion injury is expected to occur during open heart surgery.

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