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Continuous retrograde warm blood reperfusion reduces cardiac troponin I release after heart transplantation: a prospective randomized study

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Abstract During heart surgery, cardiac troponin I (cTn-I) measurement provides a tool to evaluate different cardioprotective techniques. To investigate myocardial protection during heart transplantation (HTx), cTn-I and creatine kinase (CK)-MB release was measured in 42 patients randomized to receving either continuous retrograde warm blood reperfusion or no reperfusion after cold cardioplegia. A significant linear correlation was found between donor heart ischemic time and peaks and the area under the curve of cTn-I and CK-MB release. In patients with an ischemic time longer than 90 min, cTn-I release was significantly lower in those receiving continuous retrograde warm cardioplegia than in controls. No significant difference was observed for CK-MB, tCK, and myoglobin. Our data suggest that the measurement of postoperative cTn-I release may provide a method to evaluate ischemic cardiac damage after HTx. When the ischemic time is longer than 90 min, warm retrograde blood cardioplegia provides better myocardial protection than no reperfusion.

Key words Troponin I · CK-MB · Creatine kinase isoenzymes · Myoglobin · Cardioplegic solution · Heart transplantation

Introduction

Several biochemical markers have been used in clinical practice for the diagnosis of myocardial damage. The creatine kinase-MB isoenzyme (CK-MB) has been a benchmark for these markers, but it is not specific for myocardium. The cardiac muscle isoform of cardiac troponin I (cTnI) is a protein uniquely expressed in the adult human heart. The determination of cTn-I compared with the conventionally applied analysis of cardiac enzymes offers several distinct advantages: cTn-I is normally not detectable in serum; it is a cardiospecific protein; and as a structurally compartmented substance, its release in serum lasts long after cell damage. Since the skeletal muscle does not express cardiac troponin, the measurement of cTnI confers to the cardiac isoform absolute specificity for the myocardium [6]. The measurement of cTnI may be particularly helpful in the assessment of myocardial damage during heart surgery and may provide a reliable tool for evaluating and comparing different cardioprotective procedures [3].

Myocardial protection during cardiac ischemia is generally achieved by cold crystalloid hyperkalemic solution administered before heart harvesting, cool storage at 4 °C, and eventually by additional topical cooling during heart implant. This approach is simple, maintains ventricular function and ultrastructural integrity, prevents depletion of high-energy substrate, and ensures a bloodless field [8]. Warm blood continuous retrograde hyperkalemic perfusion provides potentially ideal myocardial recovery and protection. The basic objectives of warm blood retrograde reperfusion are to optimize aerobic metabolism and to reduce myocardial ischemia and reperfusion-induced myocardial damage [5]. The aim of this study in heart-transplanted patients was to compare continuous retrograde warm blood cardiople-

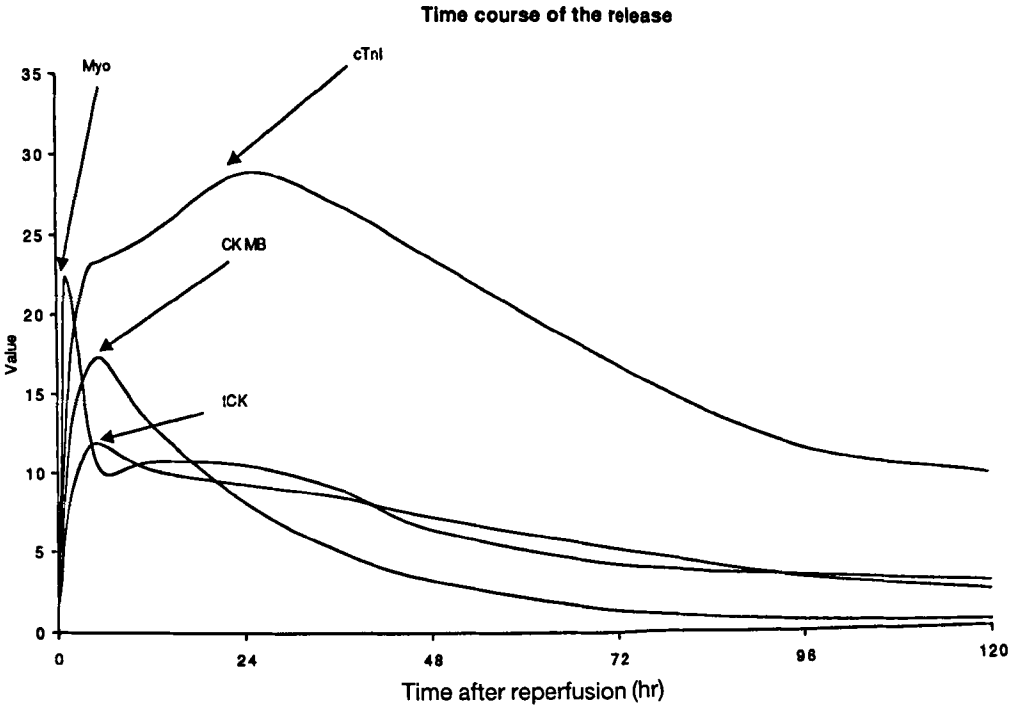

Fig. 1 Time course of the release of myocardial-specific (cardiac troponin I, *cTnI* and creatinine kinase-MB isoenzyme, *CK-MB*) and -unspecific (myoglobulin, *Myo* and total creatine kinase, *tCK*) substances after reperfusion. To obtain the *y*-axis, the value of cTnI was plotted as measured, CK-MB values were divided by a factor 10, Myo and tCK values were divided by a factor of 100

gia after heart harvesting and transportation with no reperfusion after cold cristalloid cardioplegia; we assessed cTn-I and CK-MB release as specific markers of the amount of myocardial damage induced by heart arrest and cold storage.

Patients and methods

We performed a randomized prospective study comparing retrograde warm blood reperfusion with no reperfusion after antegrade cold cristalloid cardioplegia. Fortytwo patients undergoing heart transplantation (HTx), were randomized to receving either no reperfusion or continuous retrograde warm blood cardioplegia. All hearts underwent initially antegrade cold cristalloid cardioplegia during heart harvesting in the donor. After cold storage at 4 °C during transportation, the heart was implanted immediately in the no reperfusion group (controls, $n = 26$) and myocardial perfusion was begun at aortic declamping. In the warm reperfusion group ($n = 26$), upon arrival in the operating room, a retrograde perfusion cannula was introduced into the coronary sinus, and a retrograde infusion was begun with normothermic oxygenated blood, mixed with a solution of KCl (2 mEq/ml). The retrograde reperfusion flow varied between 100 and 200 ml/min, depending on the pressure reached in the coronary sinus, wich was continuously monitored and kept below 50 mmHg during the entire procedure and discontinued at aortic declamping.

To evaluate the release of cTn-I, CK-MB mass, total CK (tCK) and myoglobin (Myo), blood samples were obtained at the following times: just before graft implantation during extracorporal circulation; at aortic declamping (time 0 or baseline); at 1, 2, 4, 6, 12, 24, 36, 48, 72, 96, and 120 h later. Venous blood for the determination of myocardial markers was collected in dry tubes, allowed to clot for 30 min at room temperature and centrifugated for 10 min. Sera were stored in aliquots at –20 °C for subsequent analysis and were frozen and thawed only once. The cTnI, CK-MB mass, and Myo were measured in all samples with a commercially available fluorometric enzyme immunoassay (Stratus; Baxter Dade, Miami, Fla.). The tCK activity was measured with an enzymatic method (Dax 24, Technicon). Normal reference values are: cTnI < 0.30 ng/ml; myoglobin < 70 ng/ml; CK-MB < 6 ng/ml; and tCK < 186 U/l.

Since most biochemical values were not normally distributed, the Wilcoxon nonparametric and Mann-Whitney-U-test were used to compare quantitative data. Qualitative data were compared using the chi-square test. Area under the curve (AUC) of the release of the four substances were calculated by the trapezoidal rule.

Results

No difference was found between the two groups regarding donor and recipient age, sex, and diagnosis at transplantation. The mean ischemic times averaged 116 ± 50 and 106 ± 43 min in the no-reperfusion and warm retrograde reperfusion groups, respectively. The time course of the release of the four substances is shown in Fig. 1: cTnI increased 7-fold 30 min after reperfusion and peaked with an increase of 43-fold at an average of 26 h later ($P = < 0.001$ vs baseline levels), remaining at high levels for 5 days after transplantation. In contrast, CK-MB, Myo, and tCK levels peaked with an increase of 47-fold, 11-fold, and 9-fold at an average of 7.7, 5.6 and 17.7 h, respectively, returning to baseline levels during the next 5 days after transplantation (Fig. 1 and Table 1).

Table 1 Release of myocardial-specific and -unspecific substances after heart transplantation (data are mean ± SE)

	Baseline	30 min after reperfusion	Peak	Time to peak (h)
cTnI (ng/ml)	0.85 ± 0.34	7.2 ± 0.7	37.8 ± 3.2	26 ± 3
CK-MB (ng/ml)	3.9 ± 1.3	42.0 ± 4.8	186.5 ± 19.6	7.7 ± 1
Myo (ng/ml)	207 ± 46	1073 ± 93	2448 ± 230	5.6 ± 1.4
tCK (U/l)	139 ± 34	344 ± 27	1385 ± 120	17.7 ± 3.9

A significant positive linear relationship was found between ischemic times and cTnI, CKMB, Myo, and tCK release at 30 min, 1, 2 and 4 h after declamping. There was also a significant linear relationship between ischemic times and the area under the curve of CK-MB release. For each variable, this relationship had an R value > 0.33 and a P value < 0.05. The relationship between ischemic times and CKMB release 30 min after reperfusion is shown in Fig. 2. Since we were interested in the protective effect of warm retrograde reperfusion against long ischemic times and controls generally showed a trend toward a greater release of myocardial specific substances, the patients were divided into locally procured hearts with an ischaemic time shorter than 90 min ($n = 16$) and distantly procured hearts ($n = 26$) with an ischaemic time longer than 90 min. In the group of patients with the longer ischemic time, the release of cTnI after reperfusion was significantly lower in warm retrograde perfusion-treated grafts ($n = 12$) than in controls ($n = 14$) at time 0, 30 min, and 1 h (1.1 ± 1.4, 5.1 ± 2.3, and 7.6 ± 4.2 vs 4.0 ± 1.8, 9.0 ± 3.9, and 17 ± 11 ng/ml for warm retrograde reperfusion and controls, respectively; $P < 0.01$) and throughout the following 5 postoperative days (AUC: 1717 ± 746 vs 2754 ± 1589 ng/ml/h, $P < 0.05$; Fig. 3). The cardioprotective effect of warm retrograde perfusion was further suggested by the finding that it resulted in a significantly ($P < 0.05$) lower CKMB release at time 0, 30 min, and 1 h (5.1 ± 6, 28 ± 15, and 61 ± 40 vs 27 ± 15; 66 ± 37, and 131 ± 92 ng/ml for warm retrograde reperfusion and controls, respectively; $P < 0.02$).

Discussion

Several papers suggest a relevant role of ischemic damage in relation to Htx morbidity and mortality. The acute ischemic damage induces activation of complement, increases expression of MHC antigens promoting both humoral and cellular rejection, increases the susceptibility to cytomegalovirus infections and the development of chronic allograft vasculopathy. Efforts to lessen ischemic peritransplant injury to the graft may decrease all these undesiderable effects. The ischemic injury may be assessed by measuring the postoperative release of myocardial specific and -unspecific substanc-

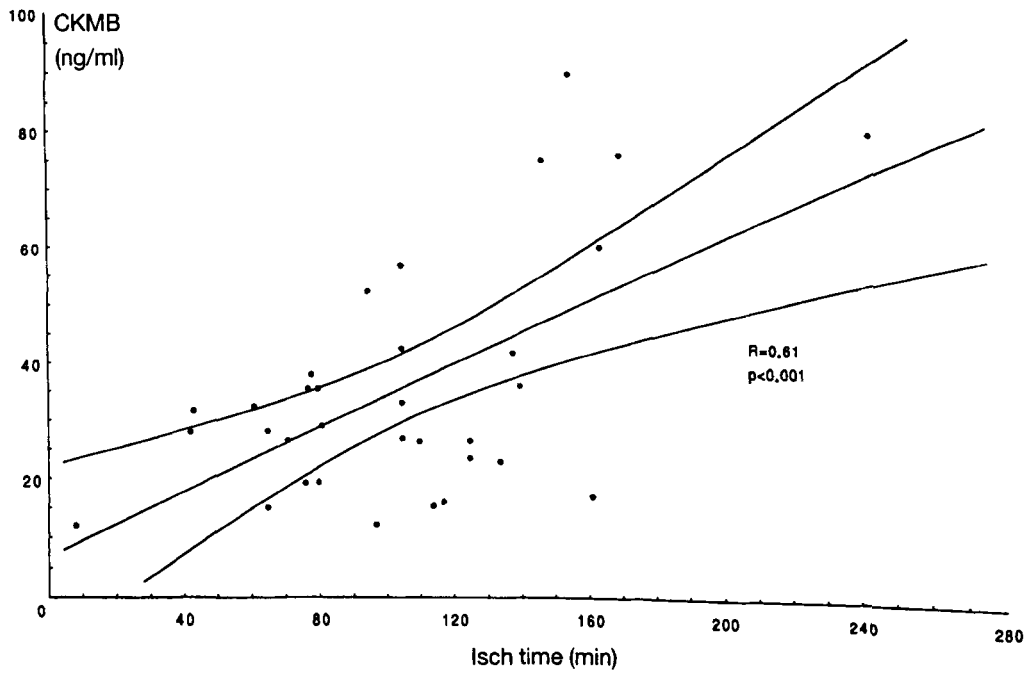

Fig. 2 Linear relationship and 95% confidence intervals between ischemic times and CK-MB release 30 min after reperfusion

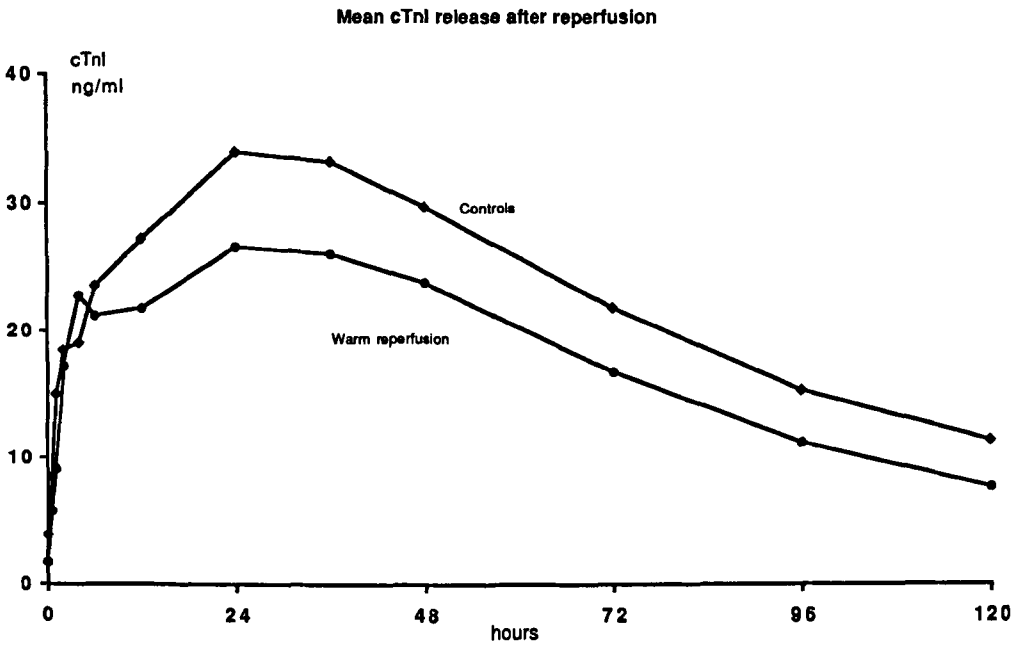

Fig. 3 Time course of the release of cTnI after warm blood retrograde reperfusion as compared with controls in patients with ischemic times longer than 90 min. A significant difference in the area under the curve of cTnI release was found ($P < 0.05$)

es. Gensini et al. [4] demonstrated that serial measurements of cTnI provide a useful tool for confirming or excluding the diagnosis of perioperative myocardial damage after conventional cardiac surgery. They suggested that cTnI evaluation can be used as a prognostic marker for patients undergoing cardiac surgery for detecting smaller myocardial infarcts. Their results were confirmed by Alyanakian et al. using cTnI measurements to detect myocardial infarction in patients undergoing cardiac surgery.

We compared myocardial protection with retrograde warm blood reperfusion versus no reperfusion with the premise that the best method would induce the lowest release of cTnI. The interest of such a comparison is highlighted by the variety of myocardial protection methods used during cardiac surgery [7] which demonstrate that there is no clear evidence to prefer one method over the others. Our results show that after heart harvesting and transportation, a large release of myocardial-specific and -unspecific substances does occurr, and the amount of cTnI and CK-MB release is significantly related to ischemic times. This finding is not surpring since Etievent et al. [3] demostrated a positive correlation between aortic cross-clamping time and cTnI release after aortic valve replacement. The positive correlation between ischemic times and cTnI or CK MB release shows these substances to be markers of myocardial ischemia or damage.

In our study, myoglobin is the first substance to be released and to reach its peak. Just as myoglobin can be considered a highly sensitive marker (first to be released), but with low specificity for myocardial tissue, cTnI is a highly specific marker, being released only by myocardial tissue, but less sensitive, as its peak of release is about 26 h from after onset of damage. It my be suggested that cTnI is too sensitive a test in Htx with an uneventful postoperative course because all patients experience a large release of this substance. However, Babatasi et al. [1] have shown that cTnI did not increase during and after coronary artery occlusion and local immobilization of the heart, suggesting that conventional cardiac surgery does not increase circulating levels of cTnI. Our data suggest that the measurement of cTnI release after Htx can be used to evaluate the amount of postoperative myocardial damage induced by heart arrest and cold storage. Cardiac TnI has a release kinetics comparable to that of tCK, but being totally myocardium specific, it may well represent myocardial cell damage. Moreover, the evaluation of tCK creates special problems due to the influence of muscle mass, exercise, gender, sex, and race. Initial elevations of cTnI (within 6–12 h) are thought to be caused by release of cTnI present in an early releasable pool. Persistent elevations are thought to be caused by slow release of cTnI complexed to the contractile apparatus. Prolonged elevations of cTnI facilitate evaluation even when blood samples are obteined days after the cardiac injury.

CK-MB release immediately after Htx (within 4 h) is significantly related to ischemic times. This finding confirms the concept that the release of myocardial-specific substances may provide a tool to evaluate ischemic damage. However, the significant relationslip between CK-MB and ischemic times is lost after the 4th h, and the area under the curve of CK-MB release was not significantly related to ischemic times. This is probably due to the fact that elevations of CK-MB do not persist as long in serum as do elevations of cTnI. Thus, cTnI measurement provides a more effective method to assess is-

chemic damage since the overall AUC of its release is significantly related to the ischemic times.

Caputo et al. [2] have used cTnI measurement to compare two different types of cardioplegia. They found that cTnI release was similar when either cold crystalloid or cold blood cardioplegia was used. They suggested that both methods offer identical protection to the myocardium in a low-risk group of patients. Our data show a significantly lower release in cTnI after warm blood retrograde reperfusion, suggesting that this technique provides better myocardial protection.

In conclusion, cold cristalloid cardioplegia may be efficient for myocardial protection during short periods of ischemic time. Cardiac TnI measurements suggested the superiority of warm blood retrograde reperfusion when ischemic times become longer. Warm retrograde perfusion may reduce the risk of ischemia-related morbidity following heart transplantation, and we suggest using warm retrograde reperfusion for cardiac recovery if the ischaemic time is longer than 90 min.

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