Different intracellular compartmentations of cardiac troponins and myosin heavy chains: a causal connection to their different early release after myocardial damage

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We investigated the net myocardial release of creatine kinase isoenzyme MB (CKMB), myoglobin, cardiac troponin T (cTnT), cardiac troponin I (cTnI), and cardiac β-type myosin heavy chain (β-MHC) into the coronary circulation after cardioplegic cardiac arrest in humans. Cardiac markers were measured in paired arterial, central venous, and coronary sinus blood in 19 patients undergoing elective coronary artery bypass grafting (CABG) before aortic cross-clamping and 1, 5, 10, and 20 min after aortic declamping. cTnT and cTnI were released into the coronary sinus in parallel to each other and almost simultaneously to myoglobin and CKMB within 20 min of reperfusion. In contrast, no β-MHC was released in the same patients during the study period. The average soluble cTnT and cTnI pools in right atrial appendages of 11 patients with right atrial and right ventricular pressures within reference values were comparable and were ~8% of total myocardial troponin content. The soluble β-MHC pool was <0.1% in all patients. Our results demonstrate the impact of the different intracellular compartmentation of regulatory and contractile proteins on their early release from damaged myocardium.

Laboratory measurement of cardiac regulatory and contractile proteins has considerably improved the diagnosis of myocardial damage caused by several diseases, such as acute and perioperative myocardial infarction, myocarditis, or heart contusion (1, 2). Troponin T and troponin I are regulatory proteins of the muscular thin filaments and part of the troponin-tropomyosin complex in striated muscle. They exist as specific isoforms, cardiac troponin T (cTnT) and cardiac troponin I (cTnI), in the myocardium (3, 4). The conventional markers creatine kinase (CK) and its MB isoenzyme (CKMB) as well as myoglobin are predominantly cytosolic proteins and not heart-specific. Cardiac β-type myosin heavy chain (β-MHC) is the predominant MHC type in human adult healthy and diseased myocardium (5). Together with the myosin light chains, β-MHC forms cardiac myosin, the major structural protein of the myocardium. Cardiac β-MHC is co-expressed in slow twitch skeletal muscle fibers and is therefore not cardiac-specific (5).

Previous clinical (1, 6–8) and experimental (9–11) studies investigated the release kinetics of myocardial macromolecules after myocardial damage. Our recent experimental observations showed that cTnT, cTnI, CK, and lactate dehydrogenase (LD) increase in parallel in effluents from isolated perfused Langendorff rat hearts after 60 min of hypoxia-induced myocardial damage (11).

In open heart surgery the aortic cross-clamping with cardioplegic cardiac arrest induces a global myocardial ischemia, and hypothermia is induced to protect the myocardium. This human model of controlled ischemia facilitates the investigation of the pathophysiological events occurring during myocardial ischemia and reperfusion in humans. Unique to our study were the direct determination of the net myocardial release of CKMB,

4 Nonstandard abbreviations: cTnT, cardiac troponin T; cTnI, cardiac troponin I; CK, creatine kinase; CKMB, creatine kinase MB isoenzyme; MHC, myosin heavy chain; β-MHC, cardiac β-type heavy chain myosin; LD, lactate dehydrogenase; and CABG, coronary artery by-pass grafting.
myoglobin, cTnT, cTnI, and β-MHC into the coronary circulation during reperfusion after cardioplegic cardiac arrest in patients undergoing elective coronary artery by-pass grafting (CABG) and the quantification of soluble cTnI, TnT, and β-MHC pools in human fresh myocardium.

**Patients and Methods**

All procedures were in accordance with the Helsinki Declaration of 1975, as revised in 1983. After institutional approval and informed consent, 19 patients undergoing elective CABG were studied (16 men, 3 women; median age, 60 years; range, 37–73 years). None of the 19 patients had clinical signs of congestive heart failure. The median preoperative left ventricular ejection fraction was 56% (range, 18–86%; <40% in three patients). A three-vessel disease was found in 14 of the 19 patients, with left main artery stenosis in 3 of them. Patients received a median of three grafts (range, 2–4). The internal mammary artery was used as a by-pass vessel in 15 patients. Perioperative myocardial infarction was diagnosed when CKMB exceeded 50 μg/L in the morning of the first postoperative day, and new Q waves developed perioperatively in at least two contiguous leads of the electrocardiogram (12).

**Anesthesia, Cardiopulmonary By-Pass Technique, and Cardioplegia**

Anesthesia was induced with 0.3–0.4 mg/kg midazolam and 5–10 μg/kg fentanyl in all patients. Endotracheal intubation was facilitated with 0.1 mg/kg vecuronium. Anesthesia was maintained using a continuous infusion of fentanyl (20 μg · kg⁻¹ · h⁻¹) and midazolam (0.15 mg · kg⁻¹ · h⁻¹). Additional bolus doses of fentanyl and isoflurane (<1.5%) were administered according to clinical requirements. All patients received an initial bolus dose of aprotinin (280 mg) over a period of 15 min followed by a continuous infusion (70 mg/h).

A standard cardiopulmonary by-pass technique (roller pump, membrane oxygenator, and cardiotomy reservoir) with moderate systemic hypothermia (core temperature, 30–32 °C) was used in all patients. The extracorporeal circuit was primed with 100 mL of 200 g/L human albumin, 250 mL of 200 g/L mannitol, and Ringer’s lactate. The pump prime also contained 280 mg of aprotinin. Blood was added to the prime only when preoperative hemoglobin was <100 g/L.

After aortic and right atrial cannulation, a 14F coronary sinus retrograde perfusion catheter was inserted. Cardiopulmonary by-pass was started, and the body core temperature was reduced to 30–32 °C. Additional topical cooling of the heart was applied. The heart started to fibrillate with topical cooling in all patients, and the coronary arteries were inspected. Thereafter the aorta was cross-clamped. Myocardial protection during aortic cross-clamping was achieved by combined antegrade and retrograde, cold, multiple dose hyperkalemic cardioplegia: 1000–1200 mL of cold (6–8 °C) St. Thomas Hospital II solution was infused into the aortic root, followed by 300 mL infused via the coronary sinus perfusion catheter. After completion of each peripheral anastomosis, an additional dose of 300–500 mL of cardioplegic solution was infused through the aortic root, the venous grafts, and the coronary sinus perfusion catheter. Oxygenated blood from the by-pass circuit was added to these additional doses of cardioplegia (ratio of blood to St. Thomas solution, 1:3). Systemic rewarming was started while the final peripheral anastomosis was performed. After aortic declamping, high by-pass flows were maintained to completely unload the beating heart and prevent it from ejecting blood during reperfusion.

**Blood Sampling and Laboratory Analysis**

A single central venous blood sample (baseline sample) was obtained immediately after induction of anesthesia. Additional paired arterial, central venous, and coronary sinus blood samples were obtained after atrial cannulation before aortic cross-clamping (pres ischemic sample) and 1, 5, 10, and 20 min after aortic declamping (reperfusion samples). Blood was collected from the arterial line of the by-pass circuit (arterial sample), from the central venous catheter (central venous sample; correct position of the catheter in the central venous circulation was assured by chest x-ray), and from the pressure monitoring line of the coronary sinus perfusion catheter (coronary sinus sample; correct position of the perfusion catheter was assured by the surgeon each time before blood was sampled).

All blood samples were assayed for lactate, cTnT, cTnI, CKMB mass, myoglobin, and cardiac β-MHC. Lactate was determined without delay. Blood samples for cTnT, cTnI, CKMB mass, myoglobin, and β-MHC measurements were centrifuged immediately (2000g for 15 min), and the plasma was frozen and stored at −20 °C until analysis. To adjust for hemodilution during by-pass, the results of cTnT, cTnI, CKMB mass, myoglobin, and β-MHC were expressed per gram of total serum protein. Total protein concentrations were measured by the Biuret method (Merck). The reference interval is 67–87 g/L.

**LD and lactate.** LD activity and lactate concentrations were determined enzymatically (Boehringer Mannheim). Myocardial lactate production was calculated as the coronary sinus lactate concentration minus the arterial lactate concentration. The cumulative myocardial lactate production during reperfusion was calculated as the mean of all four measurements during reperfusion.

**cTnT and cTnI.** Commercially available enzyme immunoassays developed by Katus et al. (13) (Boehringer Mannheim) and by Larue et al. (14) (ERIA Diagnostics Pasteur) were used for cTnT and cTnI determination, respectively. The upper limit of the reference interval for the cTnT assay used was 0.2 μg/L (13). The enzyme immunometric...
assay used for cTnI measurements showed no cross-reactivity with skeletal muscle troponin I or other cardiac proteins (14). The upper limit of the reference interval for cTnI is 0.1 µg/L.

**CKMB.** CKMB mass concentration was measured by a microparticle enzyme immunoassay (Abbott) for use with the Abbott IMx automated analyzer (15). The upper limit of the reference interval is 70 µg/L (16).

**β-MHC.** Cardiac β-MHC plasma concentrations were measured by an immunoradiometric assay (ERIA Diagnostics Pasteur) developed by Larue et al. (17). Because of the strong structural similarity of cardiac β-MHC and MHC of slow-twitch skeletal muscle fibers, this assay cross-reacts strongly with human slow-twitch skeletal MHC. The upper limit of the reference interval is 400 microunits/L; one microunit/L corresponds to 1 µg/L (17).

The myocardial release of cardiac markers (CKMB, myoglobin, cTnT, cTnI, and β-MHC) was calculated as the coronary sinus concentration minus the corresponding arterial concentration at the same measuring time point. Cumulative cardiac marker release was calculated as the mean of net release at all four measuring time points during reperfusion.

**DETERMINATION OF THE SOLUBLE cTnT, cTnI, AND β-MHC POOL IN ATRIAL TISSUE SPECIMENS**

At our institution, right atrial appendages are excised as part of the routine surgical procedure during cannulation in CABG patients when a standard cardiopulmonary by-pass technique is used during heart surgery. To avoid a possible bias from hemodynamic overload of the right heart, only right atrial appendages from 11 patients with right ventricular and right atrial pressures within reference values were used to determine the soluble cTnT, cTnI, and β-MHC pools in fresh myocardium. After excision, the tissue was immediately rinsed in ice-cold cardioplegic solution, shock-frozen in liquid nitrogen, and stored at −80°C until further analysis.

The sarcoplasmatic and structurally bound fractions of cardiac regulatory and contractile proteins were determined according to a previously published protocol (6, 18). Briefly, the tissue was homogenized in a buffer (0.05 mol/L Tris hydrochloride, 2 mmol/L EDTA, and 0.5 mmol/L dithiothreitol, pH 7.0) and stirred at 4°C for 1 h. Thereafter the insoluble molecules were sedimented by ultracentrifugation (1 h, 100 000g, 4°C). The pellet was washed, and centrifugation was repeated. Troponins were extracted from insoluble myofibrils by homogenization of the precipitate in buffer containing 0.4 mol/L potassium chloride, 0.1 mol/L potassium dihydrogen phosphate, 0.05 mol/L dipotassium hydrogen phosphate, 0.04 mol/L sodium pyrophosphate, and 0.01 mol/L magnesium chloride, pH 7.0, and stirred for 1 h at 4°C. The solubilized troponin complex was separated from insoluble actomyosin and cellular debris by centrifugation (1 h, 20000g, 4°C). This extraction step was repeated once. The remaining pellet with actin-myosin filaments was resuspended in 50 mmol/L sodium pyrophosphate (pH 7.4) for β-MHC measurements. The LD, CKMB, myoglobin, troponin, and β-MHC concentrations were measured in the soluble and bound fractions. LD, a soluble cytosolic enzyme, was used to check the quality of fraction separation by the centrifugation protocol used. The efficacy of this protocol for troponin solubilization has been documented previously (6). The cytosolic fraction was divided by the total fraction (cytosolic plus myofibrillar) to obtain the percentage of the measured markers in the cytosolic fractions.

**STATISTICS**

Cumulative myocardial lactate production and cardiac marker release were calculated as the mean of all four measurements during reperfusion according to Matthews et al. (19). The medians, ranges, and interquartile ranges were calculated to describe continuous variables. Spearman rank correlation coefficients were calculated to describe the association between variables. Nonparametric analysis of variance (Friedman two-way ANOVA) and the Wilcoxon matched pairs signed-ranks test were used for statistical analysis. P values <0.05 were considered significant.

**Results**

None of the 19 patients fulfilled the criteria for diagnosing perioperative myocardial infarction, and none of the patients suffered postby-pass cardiac failure. All were easily weaned from extracorporal circulation with a dose of dopamine of <5 µg · kg⁻¹ · min⁻¹. The median by-pass time was 104 min (range, 56–181 min). The median aortic cross-clamping time was 54 min (range, 24–84 min).

Moderate myocardial ischemia during aortic cross-clamping was indicated by a myocardial production of lactate immediately after aortic declamping (median, 34 mg/L; range, 2.0–77.0 mg/L 1 min after aortic declamping). There were no significant correlations between aortic cross-clamping times and cumulative cardiac marker release. However, we found moderately significant correlations between cumulative lactate production and cumulative cardiac marker net release (myoglobin: r = 0.49, P = 0.037; CKMB: r = 0.71, P = 0.002; cTnI: r = 0.54, P = 0.017; cTnT: r = 0.76, P = 0.0001).

**MYOGLOBIN, CKMB MASS, cTnT, AND cTnI**

Intraoperatively, the concentrations of myoglobin, CKMB mass, cTnT, and cTnI rose significantly (P <0.0004) within 20 min of reperfusion compared with the central venous baseline values obtained immediately after induction of anesthesia. Myoglobin, CKMB mass, cTnT, and cTnI
markedly exceeded their upper reference limits. The highest concentrations of all four analytes were measured in the coronary sinus (from 18-fold to 62-fold above the upper reference limit) after aortic declamping (Figs. 1–4). Coronary sinus myoglobin and CKMB mass concentrations were significantly ($P < 0.039$) higher than corresponding arterial concentrations in all four paired blood samples obtained after aortic declamping, indicating myoglobin and CKMB mass release from the human heart. There was no release of myoglobin and CKMB before aortic cross-clamping, and in contrast, there was considerable release of both markers within 20 min after aortic declamping (Figs. 1 and 2). These arterial coronary sinus myoglobin and CKMB mass concentration differences showed significant ($P < 0.014$) increases immediately (with the first minute) after aortic declamping compared with baseline values before aortic cross-clamping, which indicated a very rapid release from the myocardium with the onset of reperfusion.

The concentrations of coronary sinus cTnT and cTnI...
after aortic declamping were also higher than the corresponding arterial concentrations, indicating considerable myocardial net release of troponins within 20 min after reperfusion (Figs. 3 and 4). Five, 10, and 20 min after aortic declamping, myocardial cTnT and cTnI release was significantly ($P < 0.033$) higher compared with baseline values before aortic cross-clamping. In contrast to myoglobin and CKMB, the difference among baseline and release values for cTnT and cTnI at 1 min after aortic declamping were not significant.

**CARDIAC β-MHC**

We found no significant increase in β-MHC compared with baseline values within the first 20 min of reperfusion after hypothermic cardioplegic cardiac arrest during CABG. In all 19 patients, β-MHC concentrations did not increase over the upper limit of the reference interval during our observation period. The highest value measured (157 microunits/L) was found in the coronary sinus blood of one patient 20 min after reperfusion, which was still clearly within the reference interval.

Fig. 3. cTnT concentrations in arterial, central venous, and coronary sinus blood during reperfusion after cardioplegic cardiac arrest in 19 uneventful patients. Concentrations are given per gram of total serum protein. Data given as median (bars) and interquartile range (IQR, error bars). HLM, heart-lung machine; ACC, aortic cross-clamping; AUC, aortic unclamping. *, significantly higher than corresponding arterial HLM cTnT concentration.

Fig. 4. cTnI concentrations in arterial, central venous, and coronary sinus blood during reperfusion after cardioplegic cardiac arrest in 19 uneventful patients. Concentrations are given per gram of total serum protein. Data given as median (bars) and interquartile range (IQR, error bars). HLM, heart-lung machine; ACC, aortic cross-clamping; AUC, aortic unclamping. *, significantly higher than corresponding arterial HLM cTnI concentration.
QUANTIFICATION OF SOLUBLE TROPTONIN AND MYOSIN POOLS

We found a considerable amount of unbound troponins in the cytoplasma of myocardium, whereas the soluble β-MHC pool was negligible when compared with the total MHC content (Table 1).

**Discussion**

The present study investigated the net myocardial release kinetics of cardiac markers during reperfusion after hypothermic cardiopleic cardiac arrest in CABG patients. The novel and unique approach of this study was to measure these cardiac markers in parallel in central venous, coronary sinus, and arterial blood samples. In our study population, we found significant myocardial release of myoglobin, CKMB mass, cTnT, and cTnI into the coronary circulation after aortic declamping within 20 min of reperfusion. The myocardial net release of CKMB, myoglobin, and cardiac troponins indicates that myocardial damage occurred during aortic cross-clamping and cardioplegic cardiac arrest. However, we found no increase in β-MHC within the first 20 min of reperfusion in the same patients, and the plasma concentrations of β-MHC clearly stayed within reference values in all patients during the study period. The time courses of myocardial myoglobin, CKMB mass, cTnT, and cTnI release were similar but not completely identical. In contrast to CKMB and myoglobin, which both showed a significant myocardial release as early as 1 min after aortic declamping, the release of troponins was significantly higher compared with baseline values starting at 5 min after aortic declamping, although troponins also started to be released from myocardium with aortic declamping. These data obtained during CABG are in accordance with our recent clinical observation of roughly equivalent early sensitivities of cTnT, cTnI, myoglobin, and CKMB mass after myocardial infarction (6) and the clinical observation by Apple et al. (20) of similar early release kinetics of cTnI, cTnT, myoglobin, and CKMB in the systemic circulation in nine patients with acute myocardial infarction and complete reperfusion of the infarct-related coronary artery. However, the 30-min increase rates for CKMB and myoglobin in this study were significantly higher than for either cTnI or cTnT, which is explained very well by our findings.

Also unique to our study is the quantification of the soluble troponins and β-MHC pools in fresh human myocardium, which revealed no significant amount of β-MHC in the cytoplasma but did reveal small cytosolic troponin pools in right atrial appendages that were removed for cannulation as a part of the routine surgical procedure. The amounts of soluble TnT and TnI (in percentage of total troponin content) were comparable. Although it can be expected that cTnT and cTnI are present in equimolar concentrations in myocardial tissue, we found marked differences when cytosolic pools were calculated as µg/g wet weight of myocardium. These differences cannot be explained by differences in troponin molecular masses. They are because of differences in troponin assay standardization; consequently the numeric results of different troponin assays may differ substantially.

This study is the first to exclude the presence of a significant soluble β-MHC fraction in the cytoplasma of human myocardium. A possible limitation is that myocardial tissue from right atrial appendages may not be representative of ventricular myocardium; however, we could test only atrial appendages for obvious ethical reasons. Our results confirm earlier reports on the presence of significant soluble troponin pools in the cytoplasma of human ventricular myocardium obtained at autopsy (6, 18). The percentages of soluble troponin pools in atrial tissue were in the range of the values found in these previous studies; in addition, the values of troponin and MHC pools were in agreement with the results from fresh left ventricular myocardium of a heart that was explanted but finally not used for transplantation (unpublished results).

Our study obviously demonstrates the impact of the intracellular compartmentation of a molecule on the rate of its increases after myocardial damage. Myoglobin and CKMB are cytosolic or predominantly cytosolic molecules, which was confirmed by our results. They are released somewhat more rapidly than regulatory proteins after myocardial damage. cTnT and cTnI are released in parallel to each other. Both troponins are mainly structurally bound and have comparably large soluble pools in myocardium. The differences in myocardial release of cardiac troponins and MHC after cardioplegic cardiac arrest in association with aortic cross-clamping is striking and can be explained very well by their different intracellular compartmentations. It is very likely that the cytosolic pools of cTnT and cTnI account for the rapid early myocardial release in parallel to myoglobin and CKMB during reperfusion of the myocardium. Our study period was too short to detect β-MHC release from the human heart. Blood sampling was limited to 20 min after aortic declamping because the coronary sinus perfusion catheter had to be removed after this time point. We therefore

**Table 1. Intracellular compartmentation of cardiac markers in fresh human myocardium.**

<table>
<thead>
<tr>
<th>Cytosolic pool</th>
<th>Per g of wet weight</th>
<th>% of total content</th>
</tr>
</thead>
<tbody>
<tr>
<td>cTnI</td>
<td>1027 µg (417–1265)</td>
<td>8.3 (4.5–10.9)</td>
</tr>
<tr>
<td>cTnT</td>
<td>278 µg (194–396)</td>
<td>7.5 (4.3–10.6)</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>973 µg (623–1606)</td>
<td>98 (96–99)</td>
</tr>
<tr>
<td>CKMB</td>
<td>111 µg (62–228)</td>
<td>91 (78–94)</td>
</tr>
<tr>
<td>β-MHC</td>
<td>670 milliunits (410–930)</td>
<td>&lt;0.1 in all</td>
</tr>
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</table>

* Data of 11 right atrial appendages from hearts with right ventricular and right atrial pressures within reference values. Appendages were excised during surgery as part of the routine procedure.

* Data given as median and interquartile range (in parentheses).
cannot comment on the release of β-MHC into the coronary circulation thereafter. In previous studies, β-MHC also increased after CABG in uncomplicated CABG patients but not before the first day after surgery (1, 21).

Our study results in humans confirm previous experimental results (9–11) that suggested that the intracellular compartmentation of a molecule has a great impact on the rate at which it is released after myocardial damage. In isolated perfused rat hearts, cTnT increased in parallel to CK and LD after global ischemia, reoxygenation, or the calcium paradox (9, 10). A simultaneous release of cTnT, cTnI, LD, and CK was also found in isolated perfused Langendorff rat hearts during reoxygenation after 60 min of hypoxia (11). Compared with these experimental studies, the extent of myocardial damage in the patients we studied was small because several cardioprotective measures are routinely used to minimize myocardial damage during cardiac arrest in heart surgery, and no patient sustained a perioperative myocardial infarction. However, myocardial ischemia occurred during CABG, which was indicated by myocardial lactate production, although none of the 19 patients fulfilled the criteria for diagnosing perioperative myocardial infarction. There were moderately significant correlations between cumulative release of myoglobin, CKMB, and cardiac troponins and cumulative myocardial lactate production, which is an excellent measure for myocardial ischemia. In contrast to experimental studies, global myocardial ischemia might not be the only cause of myocardial protein release in our patients. The mechanical trauma to the heart during CABG is very small; however, right atriotomy was performed in all of our patients for cannulation of the great vessels. Despite the small myocardial mass of the right atrium, this may also contribute to a release of myocardial macromolecules and may have negatively impaired correlations among myocardial lactate production and cardiac marker release.

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References