Sialic acid (SA), a family of acetylated derivatives of neuraminic acid, is elevated in patients with coronary heart disease. Cardiac troponin T (cTnT), myoglobin (Mb), and creatine kinase-MB (CK-MB) are specific markers of myocardial injury and are, at present, widely used to detect perioperative myocardial damage during coronary artery bypass grafting (CABG) surgery. The present study investigated the net myocardial release of SA and the cardiac markers (cTnT, Mb, CK-MB) during reperfusion after hypothermic cardioplegic cardiac arrest in 25 patients undergoing elective CABG. Additional paired arterial, central venous, and coronary sinus blood samples were obtained after atrial cannulation before aortic cross-clamping (preischemic sample) and at 1 and 10 min after aortic declamping (reperfusion samples). There were no increase in the SA, cTnT, Mb and CK-MB concentrations before aortic cross-clamping, but there was considerable release of these markers within 10 min after aortic declamping: cTnT release was significantly higher compared with baseline values before aortic cross-clamping. In contrast to SA, Mb, and CK-MB, the difference between baseline and release values for cTnT at 1 min after aortic declamping was not significant. The rate of increase for SA was significantly higher than for Mb, CK-MB and cTnT. SA is a unique and novel marker that could be particularly useful in assessing myocardial cell damage in patients undergoing cardiac surgery. (Circ J 2002; 66: 1019–1023)

Key Words: Cardiac myoglobin; Coronary artery bypass grafting; Creatine kinase; Sialic acid; Troponin T

Methods

Patients

Twenty-five patients (19 male; median age, 58 years; range, 35–71 years) undergoing elective CABG were included in the study. All procedures were in accordance with the Helsinki Declaration of 1975, revised in 1983. The study was approved by the local ethics committee. Exclusion criteria were reoperations, myocardial infarction, preoperative poor left ventricular function (ejection fraction <25%, left ventricular end-diastolic pressure >20mmHg). The internal mammary artery was used as the bypass vessel in all patients. Three-vessel disease was found in 16 of the 25 patients, with left main artery stenosis in 5 of them. Patients received a median of 3 grafts (range, 2–4). Perioperative myocardial infarction was diagnosed when CK-MB exceeded 50μg/L on the morning of the first postoperative day, and new Q waves developed perioperatively in at least 2 contiguous leads of the ECG.

Anesthesia, Cardiopulmonary Bypass Technique, and Cardioplegia

Patients were monitored with standard ECG, pulse oxymetry, esophageal temperature probe, pulmonary artery thermodilution catheter, and right internal jugular vein and left radial arterial catheterization. Patients were premedicated with diazepam (0.07 mg/kg) 30 min before anesthesia. Anesthesia was induced with thiopental (2–4 mg/kg) and fentanyl (5 mg/kg) and pancuronium (0.1 mg/kg) was used as muscle relaxant. Patients were ventilated with 50% nitrous oxide in oxygen until shortly before beginning cardiopulmonary bypass (CPB) and then nitrous oxide was discontinued. Fentanyl and isoflurane were used to maintain anesthesia. Pancuronium was administered when necessary.
The same surgical techniques were used for all operations. The pump was primed with 2 L lactated Ringer's solution, to which 50 mmol sodium bicarbonate and 2,000,000 U aprotinin were added.

Moderate systemic hypothermia (28–30˚C), using cold blood potassium cardioplegic solution and topical cooling with iced saline, was used to induce and maintain myocardial arrest. Myocardial protection during aortic cross-clamping was achieved with antegrade cold, multiple dose hyperkalemic cardioplegia: 1,000–1,200 ml of cold (6–8˚C) St Thomas Hospital II solution infused through the aortic root. After completion of each peripheral anastomosis, an additional dose of 300–500 ml of cardioplegic solution was infused through the aortic root. 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 bypass flows were maintained to completely unload the beating heart and prevent it from ejecting blood during reperfusion.

A membrane oxygenator with arterial line filter (40 µ) was used in all cases. During CPB, the hematocrit value was maintained between 18% and 25%, pump flow between 2.2 and 2.4 L·min–1·m–2, and mean aortic pressure was maintained between 18% and 25%, pump flow was used in all cases. During CPB, the hematocrit value clamping; AUC, aortic unclamping. *Significantly higher than corresponding arterial sialic acid concentration.

Blood Sampling and Laboratory Analysis

A single central venous and arterial 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 (preischemic sample) and at 1 and 10 min after aortic declamping (reperfusion samples). Blood was collected from the left radial artery (arterial sample), from the central venous catheter (central venous sample; the correct position of the catheter in the central venous circulation was ascertained by chest X-ray), and from the pressure monitoring line of the coronary sinus perfusion catheter (coronary sinus sample; correct position ascertained by the surgeon each time before blood was sampled).

All blood samples were assayed for lactate, cTnT, CK-MB mass, Mb, and SA. Blood samples for cTnT, CK-MB mass, and Mb measurements were centrifuged immediately (2,000 g for 15 min), and the plasma was frozen and stored at –20˚C until analysis. SA and lactate were determined without delay. To adjust for hemodilution during by-pass, the results of cTnT, CK-MB mass, Mb, and SA 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.

An enzymatic assay was used for the assessment of serum SA. All SA measurements reflected the total (free and bound) concentration. Serum lactate, cTnT and CK determinations were done by means of test kits (Boehringer Mannheim GmbH, Mannheim, Germany). The upper limit of the reference interval for the CK-MB, and Mb assays were 50 g/L, and 70 g/L, respectively. The cutoffs in this study were 600 g/L for SA with 100% sensitivity and 96% specificity, and 0.2 g/L for cTnT with 100% sensitivity and 93% specificity.

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 4 measurements during reperfusion. The myocardial release of SA and each of the cardiac markers (CK-MB, Mb, and cTnT) 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 4 measuring time points during reperfusion.

Statistical Analysis

Cumulative myocardial lactate production and cardiac marker release were calculated as the mean of all 4 measurements during reperfusion according to the method of Matthews et al. 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
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declamping within 1 min (p<0.015) and 10 min (p<0.013) than the corresponding arterial concentration after aortic (Figs 2, 3).

Mb and CK-MB mass release from the human heart (p<0.025) and 10 min (p<0.022) of reperfusion, indicating trival concentrations after aortic declamping within 1 min MB were significantly higher than their corresponding arterial coronary concentrations of Mb and CK-MB, but there was considerable release of measured in the coronary sinus after aortic declamping.

The highest concentrations of SA, Mb, CK-MB, and cTnT were markedly exceeded their upper reference limits. The induction of anesthesia. SA, Mb, CK-MB, and cTnT, trival venous baseline values obtained immediately after 10 min of reperfusion compared with the arterial and central venous blood during reperfusion after cardioplegic cardiac arrest.

Concentrations are given per gram of total serum protein. Data given as median (bars). Arterial, radial artery; ACC, aortic cross-clamping; AUC, arterial unclamping. *Significantly higher than corresponding arterial CK-MB concentration.

Wilcoxon matched pairs signed-ranks test were used for statistical analysis. A p value less than 0.05 was considered significant.

Results

There was no recorded cases of mortality or morbidity in this group of patients. None of the 25 patients fulfilled the criteria for diagnosing perioperative myocardial infarction, and none suffered post bypass cardiac failure. All were easily weaned from extracorporeal circulation with a dose of dopamine (<5μg·kg⁻¹·min⁻¹). The median bypass time was 115 min (range, 59–171) and the median aortic cross-clamping time was 59 min (range, 25–81).

Moderate myocardial ischemia during aortic cross-clamping was indicated by myocardial production of lactate immediately after aortic declamping (median, 32 mg/L; range, 2.7–81.1 mg/L 1 min after aortic declamping). There was no significant correlation between aortic cross-clamping time and release of SA, Mb, CK-MB and cTnT. However, we found moderately significant correlations between cumulative lactate production and SA, Mb, CK-MB, and cTnT net release (SA: r=0.74, p=0.0002, myoglobin: r=0.51, p=0.029; CK-MB: r=0.67, p=0.005; cTnT: r=0.69, p=0.003).

Intraoperatively, the concentrations of SA, myoglobin, CK-MB, and cTnT rose significantly (p<0.0005) within 10 min of reperfusion compared with the arterial and central venous baseline values obtained immediately after induction of anesthesia. SA, Mb, CK-MB, and cTnT, markedly exceeded their upper reference limits. The highest concentrations of SA, Mb, CK-MB, and cTnT were measured in the coronary sinus after aortic declamping.

There was no release of SA, Mb and CK-MB before aortic cross-clamping, but there was considerable release of SA, Mb and CK-MB within 1 min and 10 min of aortic declamping. Coronary sinus concentrations of Mb and CK-MB were significantly higher than their corresponding arterial concentrations after aortic declamping within 1 min (p<0.025) and 10 min (p<0.022) of reperfusion, indicating Mb and CK-MB mass release from the human heart (Figs 2, 3).

Coronary sinus SA concentration was significantly higher than the corresponding arterial concentration after aortic declamping within 1 min (p<0.015) and 10 min (p<0.013) of reperfusion (Fig 1). This difference in the arterial coronary sinus SA concentration showed significant 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.

Coronary sinus cTnT concentration was higher than the corresponding arterial concentration at 1 and 10 min after aortic declamping. Ten minutes after aortic declamping, myocardial cTnT release was significantly (p<0.019) higher compared with the baseline values before aortic cross-clamping (Fig 4). In contrast to SA, Mb and CK-MB, the difference between the baseline and release values for cTnT at 1 min after aortic declamping was not significant.

Discussion

Despite well-established procedures, such as hypothermia and cardioplegia, perioperative myocardial infarction during CPB still occurs, although its reported prevalence depends on the tests and diagnostic criteria used. No standards are widely accepted. There has been considerable interest in the measurement of serum SA recently, since it was found to correlate with increased ischemic cardiovascular disease. The present study investigated the net myocardial release of SA and other cardiac markers during reperfusion after hypothermic cardioplegic cardiac cardiac arrest in CABG patients. The novel and unique approach of this study was to measure these cardiac markers and SA in parallel in central venous, coronary sinus, and arterial blood samples.

During open heart surgery, aortic cross-clamping with cardioplegic cardiac arrest induces 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. Aortic cross-clamping and cardioplegic arrest during surgery induces anaerobic myocardial metabolism with an increase in net production of lactate. In patients undergoing coronary bypass surgery, we observe lactate release during reperfusion, suggesting a delayed recovery of normal aerobic metabolism. Our finding largely reflects the washout of metabolites, which accumulate during CPB, rather than continued anaerobic myocardial activity.
Myocardial ischemia occurred during CABG, which was indicated by the myocardial lactate production, although none of the present 25 patients fulfilled the criteria for diagnosing perioperative myocardial infarction. There were moderately significant correlations between the cumulative release of SA, Mb, CK-MB, and cTnT and cumulative myocardial lactate production, which is an excellent measure of myocardial ischemia. In addition, a correlation was seen between the metabolic changes and SA levels during myocardial arrest.

For diagnosis of perioperative myocardial infarction, changes in the plasma concentrations of CK-MB, Mb, and cTnT are generally measured in association with analysis of the ECG or myocardial scintigraphy.\textsuperscript{11–13,18} cTnT, which is a cardiac-specific protein located in the thin filament of the myocardial contractile apparatus, is a specific marker of myocardial ischemia and necrosis.\textsuperscript{20} The difference in amino acid composition between cardiac and skeletal muscle cTnT allows the differentiation of the 2 molecules by immunologic technique. Thus the serum level of cTnT is a useful and precise tool for diagnosis of ischemia and for assessment of myocardial damage during cardiac surgery.\textsuperscript{13,14,21}

We found significant myocardial release of Mb, CK-MB mass, cTnT, and SA into the coronary circulation after aortic declamping within 10 min of reperfusion. The net myocardial release of CK-MB, myoglobin, and cardiac troponins indicates that myocardial damage occurred during aortic cross-clamping and cardioplegic cardiac arrest. The sensitivity and specificity of cTnT was more than that of CK-MB, and myoglobin could be used as a routine indicator for myocardial protection.\textsuperscript{13,14,21} In our study, we found significant myocardial release of myoglobin and CK-MB into the coronary circulation after aortic declamping within 1 min of reperfusion; however, we did not find a significant myocardial release in cTnT within the first minute of reperfusion in the same patients.

C-TnT, Mb, and CK-MB are specific markers of myocardial injury and are, at present, widely used to detect perioperative myocardial damage during CABG surgery. However, these cardiac markers are expressed in injured striated muscles as well, which limits their specificity. Nevertheless, a few studies showed that serum SA concentrations increase in patients with acute myocardial infarction\textsuperscript{6–8} and an earlier study demonstrated that the SA content of the sarcosome from the ischemic subendocardial layer was significantly increased compared with that of the nonischemic subendocardial layer.\textsuperscript{22} An elevated concentration of serum total SA in the blood might result either from the shedding or secreting of SA from the cell membrane surface, or release of cellular SA from the cell into the bloodstream because of cell damage after myocardial injury.\textsuperscript{3,7,23}

It is known that a lot of ischemic changes occur during CABG, and the serum concentration of SA during CABG may contribute significantly to this ischemic process. In the samples taken from the artery and coronary sinus at 1 min after unclamping of aorta, early ischemic changes of CABG were seen. These differences in the SA concentration in the arterial and coronary sinus samples showed significant (p<0.015) 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. Coronary sinus SA concentration was significantly higher than the corresponding arterial concentrations at 1 min and 10 min after aortic declamping, indicating SA release from the human heart.

SA was released into the coronary sinus in parallel and almost simultaneously with cTnT, Mb and CK-MB release within 10 min of reperfusion. The time courses of SA, Mb, CK-MB, and cTnT release were similar but not completely identical. In contrast to SA, which showed a significant myocardial release as early as 1 min after aortic declamping, the release of troponins was significantly higher compared with baseline values at 10 min after aortic declamping, although troponins also started to be released from myocardium with aortic declamping.

The concentrations of coronary sinus SA, cTnT, Mb and CK-MB after aortic declamping were also higher than the corresponding arterial concentrations, indicating considerable myocardial net release of these markers after reperfusion. However, the increase rates for SA in this study was significantly higher than Mb, CK-MB and cTnT, which is explained very well by our findings. SA is a unique and novel marker, which might be particularly useful in assessing myocardial cell damage in patients undergoing cardiac surgery. However, there was little difference between the ability of SA and the other cardiac markers to diagnose myocardial damage after aortic declamping during cold cardioplegic myocardial arrest with cardioplegic solutions.

References

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