MANGANESE-CONTAINING SUPEROXIDE DISMUTASE IN BLOOD AND URINE DURING OPEN-HEART SURGERY

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Concentrations of Manganese-containing superoxide dismutase (Mn-SOD) were measured perioperatively by enzyme immunoassay in serial samples of arterial and coronary sinus blood and urine taken from 18 patients undergoing mitral valve surgery. The mean Mn-SOD concentration in the arterial blood samples was 66.2 (SD 16.1 ng/ml) at induction of anesthesia, increased gradually after reperfusion and peaked on the 2nd post-operative day [150 (SD 58.3) ng/ml]. The mean concentration of Mn-SOD in the coronary sinus blood samples was significantly higher than in the arterial samples only at the 6th hour after reperfusion [97 (SD 21.8) ng/ml vs 90.3 (SD 20.9) ng/ml, p<0.05]. Although concentrations of Mn-SOD in blood did not increase in 8 patients who underwent midline sternotomy for a mediastinal tumor, they increased dramatically in 3 patients who sustained a perioperative myocardial infarction. During open heart surgery the peak values of plasma Mn-SOD concentrations were correlated to that of plasma creatine kinase-MB concentrations (r=0.5532, n=18, p<0.05) and cardiac ischemic period (r=0.5186, n=18, p<0.05). Although the meaning of an increase in plasma Mn-SOD concentrations during open heart surgery is not clarified, it may be released from the heart and anywhere else in the body damaged during cardiopulmonary bypass.

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SUPEROXIDE dismutase (SOD; EC 1.15.1.1), is an enzyme which catalyzes the dismutation of superoxide anion radicals to hydrogen peroxide and oxygen. SODs are ubiquitous enzymes in oxygen-metabolizing cells and serve to protect against oxygen toxicity. Two different forms of SOD are generally found in the human body. One is a manganese-containing dismutase (Mn-SOD) and the other is a copper/zinc-containing dismutase (Cu, Zn-SOD). Mn-SOD is an 80-kDa tetramer found predominantly in the mitochondrial matrix, whereas Cu, Zn-SOD is a 32-kDa dimer located in the cytosol. Mn-SOD is present at a high concentration in the myocardium.

Mn-SOD is one of major inner radical scavengers and has an important role against the superoxide anion radical which is the main cause of reperfusion injury. We measured Mn-SOD concentrations in serial samples of blood and urine during open heart surgery using a recently developed highly sensitive enzyme immunoassay. It will clarify the change of blood Mn-SOD concentra-

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Mn-SOD in Open-heart Surgery

Fig. 1. Measurement protocol in the open heart surgery. Circle marks show time of arterial sampling. Square marks show time of urinary sampling. An: beginning of anesthesia. Ax: beginning of aortic cross clamping. CIP: cardiac ischemic period.

MATERIALS AND METHODS

Included in this study were 18 patients who underwent mitral valve replacement or repair. They are herein referred to as the open heart surgery group (OHS). None of these patients had associated coronary artery disease. The operations were performed through a midline sternotomy and employed hypothermic cardiopulmonary bypass with cold oxygenated crystalloid cardioplegia solution and topical cooling by ice slush. The left atrium was opened through a transseptal approach and the mitral valve was replaced with a mechanical valve.

Fig. 1 shows our sampling protocol of the OHS group. Eighteen blood samples were taken serially from a peripheral arterial line during the perioperative period. In addition, fourteen blood samples were taken from a coronary sinus line placed through the right atrium during the operation. The differences in Mn-SOD concentration between arterial blood and coronary sinus blood samples were calculated and Mn-SOD exudation from the heart was also calculated with this difference in Mn-SOD concentration and coronary sinus blood flow which was assumed to be 100 mL/min.

Each patient had an indwelling urinary catheter and urine-collection apparatus which was emptied every hour. Eleven urinary samples were also taken during the perioperative period. Urinary excretions of both Mn-SOD and creatine kinase (CK)-MB were calculated from the concentration and the urine volume.

Eight patients with a mediastinal tumor who were operated upon through a midline sternotomy comprised the control surgery group. Five blood samples were taken serially from the cubital vein during the perioperative period.

Three patients who sustained a perioperative myocardial infarction (PMI) following aorto-coronary bypass surgery were also included in this study and comprised the PMI group. Blood samples were taken under the same protocol as the open heart surgery group.

Blood samples taken from the cubital vein of 120 healthy adults were used to determine the normal range of Plasma Mn-SOD concentrations.

Plasma was separated from the heparinized blood samples by centrifugation at 1000 g for 10 min within 30 min of collection, then stored at −80 °C until analysis. Urine samples were kept at 4 °C and were analyzed within a week of collection.

Antibodies to human Mn-SOD were raised in New Zealand white rabbits by injecting Mn-SOD purified from human liver with Freund’s complete adjuvant. Antibodies to Mn-SOD were purified by immunoaffinity chromatography with Mn-SOD-coupled Sepharose 4B13–15.

Concentrations of Mn-SOD were determined with a sandwich-type enzyme immunoassay system. The assay system uses polystyrene balls (3 mm in diameter) coated
with immobilized purified antibodies to MnSOD, and the same antibodies labeled with β-D-galactosidase from Escherichia coli.\textsuperscript{12,16,17}

CK-MB concentrations were also determined by the sandwich-type enzyme immunoassay system\textsuperscript{18} and were compared with the Mn-SOD concentrations in the same sample.

Duplicate measurements were performed on each sample. Differences between samples of arterial and coronary sinus blood from each patient were evaluated by the paired t-test. Results were expressed as mean and standard deviation in the text and as mean and standard error in the figures.

RESULTS

Patient data

Included in the OHS group were 10 male and 8 female patients with a mean age of 58 (SD 8) years. Their conditions were as follows: mitral valve stenosis, 2 mitral valve regurgitation, 6 mitral valve stenosis and regurgitation, and eight mitral valve stenosis and regurgitation combined with aortic valve disease. One of them had undergone mitral valve repair, nine had undergone mitral valve replacement and the other 8 patients had undergone double valve replacement. The mean cardiac ischemic period (the period of aortic cross clamping), the cardiopulmonary bypass time and the lowest nasopharyngeal temperature were 93.5 (SD 39.2) min, 150 (SD 50) min and 24.2 (SD 2.0) °C, respectively.

Serum concentrations of Mn-SOD in healthy adults

Serum concentrations of Mn-SOD from 120 healthy adults were 77.5 (SD 18.0, range 42.3–149 ng/mL). We considered values under the mean + 4SD, 150 ng/mL, as the normal range of Mn-SOD concentrations in blood.

Perioperative changes in plasma Mn-SOD and CK-MB concentrations of the OHS group

Fig. 2 shows the plasma concentrations of Mn-SOD and CK-MB in sequential samples from the OHS group. The mean Mn-SOD concentration was 65.6 (SD 16.7) ng/ml at induction of anesthesia, decreased once to 43.1 (SD 14.7) ng/ml at the beginning of cardiopulmonary bypass because of hemodilution, increased gradually to initial levels 2 h after reperfusion, and then reached a peak of 150 (SD 58.3) ng/ml on the 2nd post-
Fig. 3. Scattergram of plasma Mn-SOD concentrations in sequential arterial and coronary sinus samples after reperfusion in the open-heart surgery group (n=18). Abbreviations are same as in Fig. 1. X axis shows time since reperfusion. Broken line shows normal range of plasma Mn-SOD concentrations. Starmark: p<0.05, arterial vs coronary sinus sample.

Fig. 4. Summation curve of Mn-SOD exudation from the heart during open heart surgery (n=18). Mn-SOD exudation was calculated with the difference of Mn-SOD concentrations between arterial and coronary sinus samples and coronary sinus blood flow which was assumed to be 100 mL/min. Data is shown as mean±1SE. X axis shows time since reperfusion.

decreased gradually thereafter. Plasma MnSOD concentrations increased more slowly and peaked later than did plasma CK-MB concentrations.

Fig. 2 also shows plasma Mn-SOD concentrations in the control surgery group. The mean Mn-SOD concentration in the control surgery group was 86.0 (SD 23.1) ng/ml at induction of anesthesia and did not change significantly thereafter [80.1 (SD 16.2) ng/ml on the 1st postoperative day].

Differences between arterial and coronary sinus plasma Mn-SOD concentrations of the OHS group

Fig. 3 is the scattergram of plasma concentrations of Mn-SOD in serial arterial and coronary sinus samples after reperfusion in the OHS group. Plasma Mn-SOD concentrations in coronary sinus samples were significantly higher than in paired arterial samples only at the 6th hour after reperfusion [97.0 (SD 21.8) vs 90.3 (SD 20.9) ng/ml, p<0.05]. Fig. 4 shows the summation curve of Mn-SOD exudation from the heart, on the assumption that coronary sinus blood flow is 100 mL/min. It increased gradually after reperfusion and its course resembles that of blood Mn-SOD concentration after reperfusion.

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Correlation between peak plasma Mn-SOD concentrations and peak plasma CK-MB concentrations or cardiac ischemic period in the OHS group.

The peak values of plasma Mn-SOD concentrations were correlated to those of plasma CK-MB concentrations \( (r=0.5532, \ n=18, \ p<0.05) \) (Fig. 5) and to the cardiac ischemic period \( (r=0.5186, \ n=18, \ p<0.05) \) (Fig. 6). Peak plasma Mn-SOD concentra-

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Fig. 5. Relationship between the peak plasma Mn-SOD concentrations and the peak plasma CK-MB concentrations \( (n=18) \). Shaded area indicates normal range of plasma Mn-SOD concentrations.

Fig. 6. Relationship between the peak plasma Mn-SOD concentrations and cardiac ischemic period \( (n=18) \). Shaded area shows normal range of plasma Mn-SOD concentrations.

Fig. 7. Perioperative urinary excretion of Mn-SOD and CK-MB during open heart surgery \( (n=18) \). Data is shown as mean±1SE. Abbreviations are same as in Fig. 1.
Fig. 8. Scattergram of plasma Mn-SOD concentrations in open heart surgery (OHS) group (n=18), perioperative myocardial infarction (PMI) group (n=3) and control surgery group (n=8). X axis shows time of sampling. Abbreviations are same as in Fig. 1. 6 hours means 6 h since reperfusion in OHS and PMI group or end of operation in control surgery group. Broken line shows normal range of plasma Mn-SOD concentrations.

It has been reported that superoxide anion radicals play an important role in the genesis of reperfusion injury and that radical scavengers may reduce the extent of reperfusion injury. Mn-SOD is a major inner radical scavenger catalyzing the dismutation of superoxide anion radicals to hydrogen peroxide and oxygen. It is a mitochondrial enzyme and presents in the myocardium at high concentration. Recently, recombined SODs derived from Escherichia coli have been tested as radical scavengers. We tried to evaluate the change of blood concentrations of inner Mn-SOD before recombined SODs are used.

In this study we measured the concentration of Mn-SOD in blood and urine samples during open-heart surgery with a highly sensitive enzyme immunoassay. We found that: (a) in the OHS group, plasma Mn-SOD concentrations increased after reperfusion and peaked to about twice the initial value on the 2nd postoperative day; (b) Mn-SOD concentrations in coronary sinus samples were significantly higher than in arterial samples at the 6th hour after reperfusion; (c) urinary excretion of Mn-SOD increased 6-fold during cardiac arrest and for several hours after reperfusion; (d) in the control surgery group, plasma Mn-SOD concentrations did not increase perioperatively; (e) in the PMI group, plasma Mn-SOD concentrations were much higher than in the OHS group.

In the OHS group Mn-SOD is released from the heart damaged by ischemia into the blood-stream. When coronary sinus blood flow is assumed to be 100 mL/min, the average total cardiac Mn-SOD exudation is calculated to be 478 mg in first 24 h after reperfusion. 478 mg of Mn-SOD may enhance about 100 mg/mL of blood Mn-SOD concentrations when the total body blood volume is assumed to be 5L. A portion of Mn-SOD in the blood-stream is released from the heart; however, Mn-SOD, a ubiquitous enzyme, is also released from anywhere in the body damaged during cardiopulmonary bypass. We have reported that serum Mn-

DISCUSSION

It has been reported that superoxide anion radicals play an important role in the genesis of reperfusion injury and that radical scavengers may reduce the extent of reperfusion injury. Mn-SOD is a major inner radical scavenger catalyzing the dismutation of superoxide anion radicals to hydrogen peroxide and oxygen. It is a mitochondrial enzyme and presents in the myocardium at high concentration. Recently, recombined SODs derived from Escherichia coli have been tested as radical scavengers. We tried to evaluate the change of blood concentrations of inner Mn-SOD before recombined SODs are used.

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SOD concentrations increase in patients with acute myocardial infarction and do not increase in patients with angina pectoris. When perioperative myocardial infarction occurs, plasma Mn-SOD concentrations increase dramatically. During PMI Mn-SOD may be released mainly from the myocardium and its plasma concentrations may increase in proportion to the extent of myocardial damage.

Mn-SOD has a molecular mass of about 80 kDa, similar to that of CK-MB. However, Mn-SOD in the blood peaks later than does CK-MB. This may be because Mn-SOD is deeply anchored in the mitochondria and must first pass through the cytosol before being released into the bloodstream. Urinary excretion of Mn-SOD increased slightly during open heart surgery. The mechanism of Mn-SOD clearance from the bloodstream is not well understood. It may be cleared in a manner similar to CK-MB by the reticuloendothelial system. Urinary excretion of Mn-SOD increased earlier than did plasma Mn-SOD concentrations. This may be due to release of Mn-SOD from the kidney. It has been reported that Mn-SOD synthesis is stimulated by the superoxide anion radical. It has also been reported that the tissue content of Mn-SOD increases at 24h after brief cardiac ischemia in the canine heart. In open heart surgery, tissue Mn-SOD content in the heart may increase after reperfusion and its increase may enhance blood Mn-SOD concentrations.

Recently, recombinant SOD has been tested as a radical scavenger during open heart surgery. It acts best as a radical scavenger when blood SOD concentrations are enhanced about 100 fold the normal value. Inner Mn-SOD, concentrations of which is enhanced only double during open heart surgery, may play little role as a radical scavenger.

The meaning of an increase in plasma Mn-SOD concentration during open heart surgery can not be clarified in this study. However, Mn-SOD may be released from the heart and anywhere in body damaged during cardiopulmonary bypass.

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