Myocardial Release of Malondialdehyde and Purine Compounds During Coronary Bypass Surgery

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Background Free radicals and lipid peroxidation have been suggested to play an important role in the pathophysiology of myocardial reperfusion injury. The purpose of the present study was to monitor myocardial malondialdehyde (MDA) production as an index of lipid peroxidation during ischemia-reperfusion sequences in patients undergoing elective coronary bypass grafting. There has been a lot of debate on the role of xanthine oxidase as a potential superoxide anion generator and thus lipid peroxidation in human myocardium. To evaluate the activity of xanthine oxidase pathway, we measured the changes in the transcardiac concentration differences in adenosine, inosine, hypoxanthine, xanthine, and uric acid.

Methods and Results The coronary sinus–aortic root differences (CS-Ao) of MDA, oxypurines, and nucleosides were measured by a recently developed ion-pairing high-performance liquid chromatographic (HPLC) method. Fifteen patients were included in the study, and 13 of them demonstrated a more than 10-fold increase in net myocardial production of MDA on intermittent reperfusion during the aortic cross-clamp period. In 2 patients, MDA was not detectable in any of the CS or Ao samples. Before aortic cross-clamping, the CS-Ao concentration differences in adenosine, inosine, hypoxanthine, xanthine, and uric acid were 0.59±0.19, 0.23±0.05, 0.89±0.36, 0.58±0.32, and 11.4±4.9 μmol/L, respectively. After aortic cross-clamping, the sum of the transcardiac differences of these compounds increased up to 2.8-fold and then gradually decreased after declamping of the aorta. There was a weak positive correlation between transcardiac concentration differences of MDA and xanthine plus uric acid ($r=.48$, $P<.01$). The postoperative functional recovery or leakage of cardiac enzymes was not affected by the level of MDA net release during the aortic cross-clamp period, however.

Conclusions We conclude that myocardial lipid peroxidation, estimated as MDA formation, is common during intermittent ischemia-reperfusion sequences in coronary bypass surgery, although some patients may be better protected. Xanthine oxidase appears to be operative in human myocardium, and free radicals generated in this reaction might also be involved in the observed lipid peroxidation process. Increased degradation of myocardial adenine nucleotides and concomitant lipid peroxidation may play a specific role in the development of reperfusion injury. In this study, however, more extensive lipid peroxidation was not associated with impaired functional recovery. (***Circulation.*** **1994;90:291-297.**)

Key Words • lipids • peroxidation • xanthine oxidase • myocardium

Reperfusion of ischemic myocardium is clinically encountered during thrombolytic treatment of acute myocardial infarction, coronary angioplasty, and bypass surgery as well as during cardiac transplantation. Although basically beneficial, reperfusion has been shown to be associated with tissue damage, development of postischemic dysfunction (stunning) of the myocardium, and reperfusion arrhythmias.[1-5] Studies with experimental animals have emphasized the role of free radicals and lipid peroxidation in the pathophysiology of reperfusion injury and myocardial stunning.[3,6-10] Reperfusion may also lead to reduction of the antioxidant defense capacity of the heart muscle.[9]

Despite the accumulating evidence that free radical formation and subsequent peroxidation of membrane lipids also occur in humans,[5,11-14] the sources of free radicals and their role in the reperfusion injury of the human myocardium have remained unclear. The role of xanthine oxidoreductase in the formation of free radicals is especially controversial.[7,15-21]

The purpose of the present study was to evaluate the activity of the xanthine oxidoreductase pathway, monitor the generation of lipid peroxidation products in the human myocardium during ischemia-reperfusion sequences encountered in coronary bypass surgery, and assess the role of lipid peroxidation in the postoperative recovery of the patients. The activity of xanthine oxidase and the extent of lipid peroxidation were evaluated on the basis of the changes in the transcardiac differences of xanthine plus uric acid and malondialdehyde (MDA), respectively. The coronary sinus–aortic root (CS-Ao) differences of purines and MDA were measured by a recently described HPLC method,[22] which detects MDA more specifically than the commonly used thiobarbituric acid (TBA) colorimetric assay. Because

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Clinical Information for Patients Undergoing Elective Coronary Bypass Grafting

Patients, n	15
Age, y	61±5
Sex, male/female	14/1
Previous infarctions: anterior/inferoposterior/location unknown, n	2/9/4
Angina pectoris: NYHA class I/II/III/IV, n	0/0/12/3
Serum cholesterol, mmol/L	6.4±1.4
Hypertension, n	10
Diabetes: diet/tablets/insulin, n	1/2/1
Ejection fraction, %	58±11
LVEDP, mm Hg	15±5
Diseased vessels: one/two/three/left main, n	0/2/13/3
Blood cardioplegia: antegrade/antegrade+retrograde, n	11/4
Cardiopulmonary bypass time, min	174±49
Aortic cross-clamp time, min	93±19
Bypasses: four/five/six/seven/eight, n	3/4/6/1/1
Bypasses per patient, n	5.5±1.1
Endarterectomy, one/two/three, n	10/1/1
Perioperative infarctions, n	1
Postpericardiotomy syndrome, n	3
Postoperative arrhythmias or conduction disturbances, n	5

NYHA indicates New York Heart Association; LVEDP, left ventricular end-diastolic pressure.

the method allows direct and simultaneous quantitation of MDA, oxypurines, and nucleosides, it offers a convenient way to study potential relations between myocardial energy state and lipid peroxidation.

Methods

Protocol

The experimental protocol was accepted by the institutional review board. Fifteen patients who were admitted to hospital for elective coronary bypass operation were included in the study, and they all gave their informed consent for the research. The clinical characteristics of the patients as well as the details of their perioperative and postoperative courses are presented in the Table. Aspirin was withdrawn 2 weeks before the operation. Dipyridamole (150 mg TID) was started 2 days before, with the last dose given on the morning of the operation.

Before the operation, a Swan-Ganz catheter was introduced through the internal jugular vein into the pulmonary artery using the Seldinger technique. All the patients were operated on by the same thoracic surgeon (J.N.). After median sternotomy, the left internal thoracic artery was dissected, and the patients were heparinized. The ascending aorta and venae cavae were cannulated. A self-inflated balloon was introduced into the CS via the right atrium, and the left ventricle was vented with a cannula introduced via the right upper pulmonary vein.

Full cardiopulmonary bypass was initiated and maintained with a capillary oxygenator (Dideco Compactflow D705) and roller pumps (Stöckert CAPS, Stöckert Instrumente). Warm high-potassium cardioplegia was started and then replaced by cold blood cardioplegia with lower potassium content after cardiac arrest, when the heart was cooled. Antegrade cardioplegia was used in all patients, and additional retrograde cardioplegia was used via the CS in four patients.

The distal anastomoses were completed in the order of importance, decided on the basis of findings in coronary angiography, except that the left internal thoracic artery and the left anterior descending coronary artery were always anastomosed last. After each distal anastomosis, cold blood cardioplegia was delivered into the Ao and vein grafts connected to a specific manifold of the aortic cardioplegia line at a speed of approximately 100 mL/min for a time period of 3 minutes. The average number of bypasses was 5.5 per patient, and endarterectomy was done in most of the patients. The average aortic cross-clamp and cardiopulmonary bypass times were 93 and 174 minutes, respectively (Table). After the last distal anastomosis, the heart was warmed, and the proximal vein graft anastomoses were completed with the aid of a tangential clamp after removal of the cross-clamp.

The recovery of the patients was followed at the intensive care unit by repeated measurements of cardiac output (cardiac index), central venous pressure, and pulmonary capillary wedge pressure. Intra-arterial blood pressure and ECG were continuously monitored. The release of the enzyme activities of the cardiac isoform of the creatine kinase (CK-MB) and α-hydroxybutyrate dehydrogenase were followed for 3 postoperative days.

Blood Sampling and Processing

Blood was collected simultaneously from the Ao and CS before cross-clamping of the aorta (13.1±1.4 minutes); during the aortic cross-clamp period after finishing the first (19.1±1.4 minutes), third (50.1±2.7 minutes), and fifth (77.3±4.6 minutes) anastomoses; and 2, 5, 15, and 30 minutes after declamping the aorta. These samples were immediately injected into heparinized tubes on ice and centrifuged; 2 mL of plasma was acidified with 0.1 mL of 60% perchloric acid; and the extracts were neutralized for the analysis of adenine nucleotide degradation products and MDA. Because preliminary data showed that the concentrations of metabolites in CS were highest between the first and second minute of the 3-minute cardioplegia delivery period after finishing the distal anastomoses, the samples were collected during that time.

The uptake and metabolism of adenosine by red blood cells are extremely rapid, and the half-life in human plasma is only 0.6 to 1.5 seconds,[23] which makes accurate measurement of plasma adenosine difficult. However, we have previously shown that the technique used here, ie, pretreatment of patients with oral dipyridamole combined with sampling into syringes containing dipyridamole (final concentration, 0.2 mmol/L), inhibits nucleoside carrier effectively and allows reliable measurements of plasma adenosine and inosine.[24,25]

Analysis of Adenine Nucleotide Derivatives and MDA

Two hundred microliters of the neutralized perchloric acid plasma extracts, after additional extraction of dipyridamole with chloroform, were filtered on a HV 0.45-μm Millipore filter and subsequently used for the direct HPLC analysis of adenosine, inosine, hypoxanthine, xanthine, uric acid, and MDA. Perchloric acid deproteinization was used to break off the Schiff's base potentially formed by interaction of MDA and amino acids or amino groups of proteins, thus allowing to quantitate the total MDA present in plasma. Isocratic separation of various compounds was obtained as described earlier[22] by using an LC-18 T 15 cm×4.6 mm 3-μm particle column (Supelco) equipped with a guard column and connected to a Jasco 980-PU dual-pump system. Chromatographic runs were monitored by a Jasco MD-910 diode array detector connected both to a PC for the data acquisition and to an integrator SIC Chromatocorder 12. Identification of the various compounds

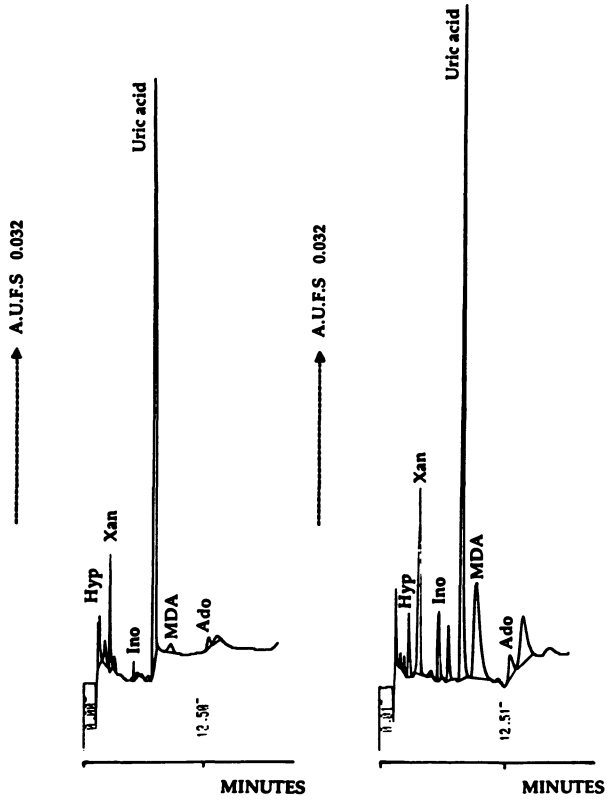

FIG 1. Chromatograms of coronary sinus (CS) plasma samples of a patient undergoing coronary bypass surgery. Left, Before aortic cross-clamp. Right, During aortic cross-clamp after the third vein graft was anastomosed. Ado indicates adenosine; A.U.F.S., absorbance units full scale; Hyp, hypoxanthine; Ino, inosine; MDA, malondialdehyde; and Xan, xanthine.

in the sample runs was performed by comparing both the retention times and the absorption spectra of each peak with peaks obtained in runs of ultrapure standards. Concentrations of the various compounds were determined by calculating the areas of each peak at 266 nm and comparing them with those obtained with runs of daily prepared standards. Examples of two HPLC chromatograms are shown in Fig 1.

Statistical Analysis

One-way ANOVA was used to test the time-dependent changes in adenosine catabolites, MDA, and indexes of postoperative pump function. The statistically significant changes ($P<.05$) were located by Duncan's multiple-range t test. The correlation between CS-Ao differences in purine compounds and MDA were analyzed with regression analysis.

Results

MDA Production During Cardiopulmonary Bypass

Thirteen patients demonstrated net myocardial production of MDA during cardiopulmonary bypass. More than 10-fold transcardiac differences in MDA were observed on intermittent reperfusion during the aortic cross-clamp period compared with the preclamp values. The CS-Ao concentration differences of MDA then decreased toward the end of the cardiopulmonary bypass period (Fig 2). In two patients, MDA was not detectable in any of the plasma samples. It is noteworthy that these patients also demonstrated constantly lower transcardiac differences in purines, with average CS-Ao differences during the observation period of

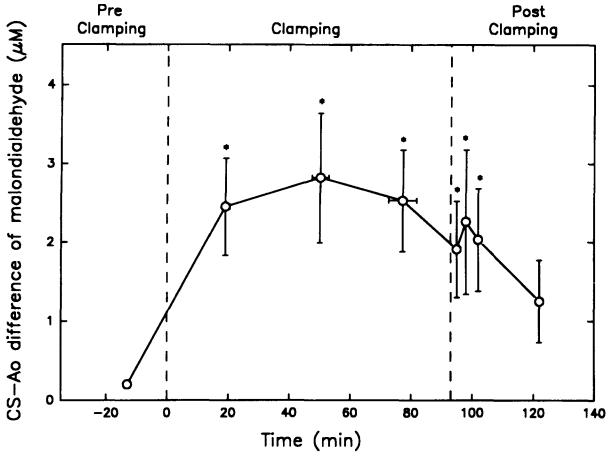

FIG 2. Plot of coronary sinus–aortic root (CS-Ao) concentration differences (μmol/L) of malondialdehyde during cardiopulmonary bypass ($P=.026$ for time-dependent changes by one-way ANOVA). The statistically significant changes (*$P<.05$ vs preclamping) were located by Duncan's multiple-range test.

10.6±6.1 μmol/L compared with 33.9±2.2 μmol/L ($P=.031$) in patients with MDA production. When the MDA producers were divided into two groups on the basis of the median value of their CS-Ao purine differences (27.5 μmol/L), it was found that those with higher transcardiac differences also had significantly higher MDA concentration differences (2.52±0.30 versus 1.05±0.21 μmol/L, $P=.002$).

Transcardiac Differences of Adenosine Metabolites

Before aortic cross-clamping, the CS-Ao differences in adenosine (0.59±0.19 μmol/L), inosine (0.23±0.05 μmol/L), hypoxanthine (0.89±0.36 μmol/L), and xanthine (0.58±0.32 μmol/L) were only slightly positive. On the other hand, the corresponding transcardiac difference in uric acid of 11.4±4.9 μmol/L indicates net uric acid production even before aortic cross-clamping (Fig 3). After cross-clamping of the aorta, progressively increasing concentrations of all adenosine degradation products were observed in the CS during intermittent delivery of blood cardioplegia solution, and the transcardiac differences in the sum of these compounds increased to 2.8-fold. Uric acid was produced most, with a transcardiac concentration difference increasing to 30.5±4.0 μmol/L. The most significant relative change in the production of adenosine compounds was observed between cross-clamping and the first distal anastomosis.

After declamping of the aorta, the transcardiac concentration differences of adenosine metabolites gradually decreased. However, at the end of the cardiopulmonary bypass, net myocardial production of purines still occurred, with uric acid composing about 80% (Fig 3).

Correlation Between Net Production of MDA and Adenosine Compounds

Xanthine oxidase catalyzing the conversion of hypoxanthine to xanthine and further to uric acid leads to production of superoxide anion.[6,7] To evaluate the potential association between the activity of this reaction sequence and lipid peroxidation, the sum of the net increases in the xanthine plus uric acid concentrations

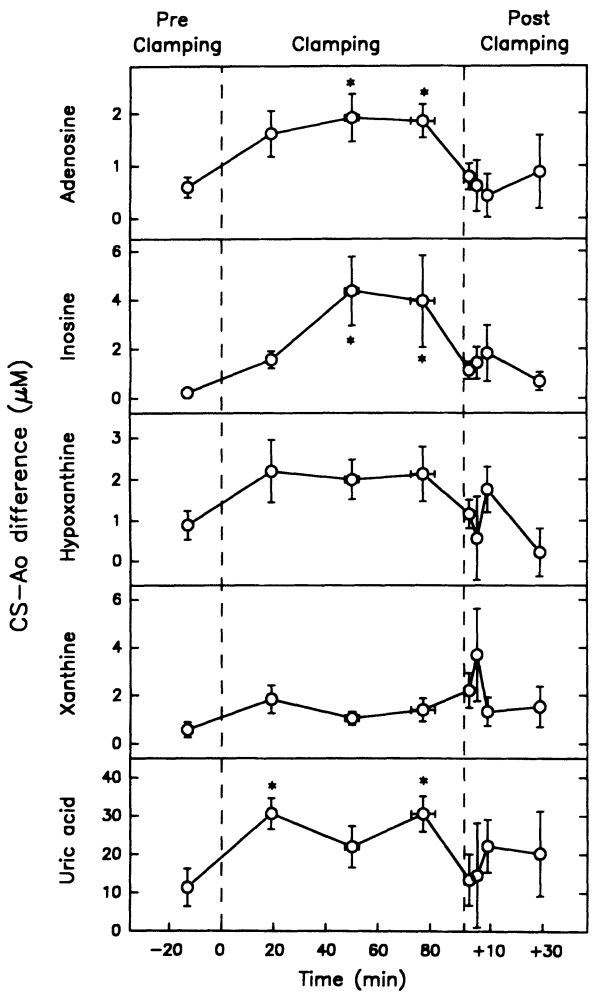

FIG 3. Plots of coronary sinus–aortic root (CS-Ao) concentration differences (μmol/L) of adenosine and its catabolites during cardiopulmonary bypass (P=.040, P=.018, P=.30, P=.18, and P=.014 for time-dependent changes of adenosine, inosine, hypoxanthine, xanthine, and uric acid, respectively, by one-way ANOVA). The statistically significant changes (*P<.05 vs pre-clamping) were located by Duncan's multiple-range t test.

were correlated with the net production of MDA (Fig 4), and a weak positive correlation was observed (r=.48, P<.01).

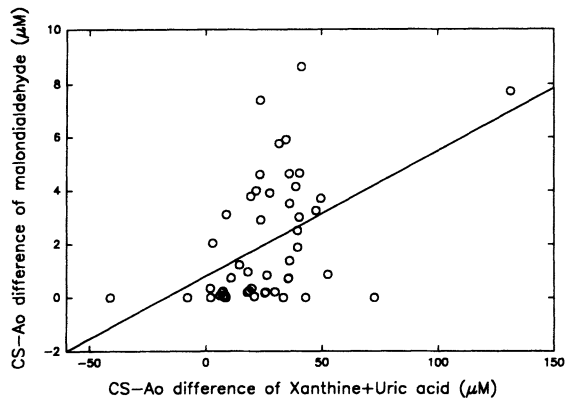

FIG 4. Scatterplot of correlation of coronary sinus–aortic root (CS-Ao) differences of malondialdehyde (μmol/L) with CS-Ao differences in xanthine plus uric acid (μmol/L) (r=.48, P<.01).

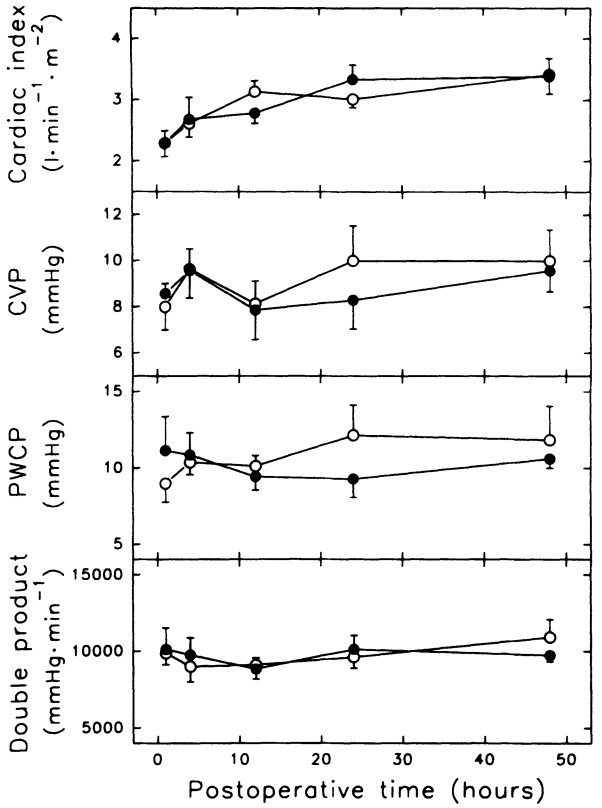

FIG 5. Plots of postoperative recovery of the study patients. Patients with mean coronary sinus–aortic root (CS-Ao) difference during aortic cross-clamp period above median (2.53 μmol/L) are indicated by closed circles and those under median by open circles. CVP indicates central venous pressure; PCWP, pulmonary capillary wedge pressure. There were no statistically significant differences between the patient groups.

Postoperative Recovery

All patients recovered from surgery despite the large number of distal anastomoses and the relatively long cardiopulmonary and aortic cross-clamp times. Intra-aortic balloon pumping device was inserted into two patients because of the prolonged need for cardiopulmonary bypass after the operations. One patient was considered to have suffered from a perioperative myocardial infarction because of the high CK-MB value on the second postoperative day (Table).

To evaluate the role of MDA in the postoperative recovery, we subdivided the patients into two subgroups on the basis of the median transcardiac difference of MDA (2.53 μmol/L) during aortic cross-clamping. It is clear from Fig 5 that there were no major differences in the indexes reflecting postoperative pump recovery in these subgroups. The efflux profiles for CK-MB and α-hydroxybutyrate dehydrogenase were also identical in the two groups (Fig 6). The mean transcardiac difference of MDA during aortic cross-clamping was somewhat higher in the patients requiring intra-aortic balloon pumping than in the rest of the patients, but the difference was not statistically significant (2.83±0.19 μmol/L versus 2.57±0.34 μmol/L).

Discussion

Direct measurement of free radicals in patients is difficult because of the transient nature of these species.

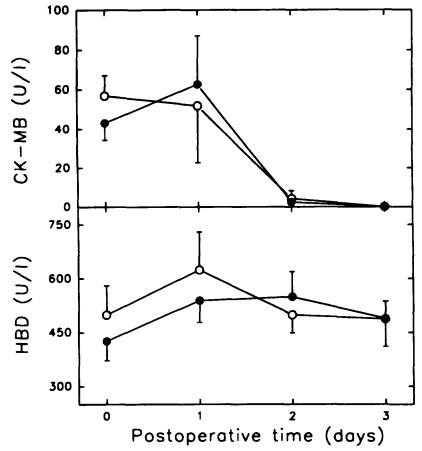

FIG 6. Plots of postoperative efflux of cardiac enzymes. Patients with mean coronary sinus–aortic root (CS-Ao) difference during aortic cross-clamp period above median (2.53 μmol/L) are indicated by closed circles and those under median by open circles. CK-MB indicates cardiac isoform of creatine kinase; HBD, α-dydroxybutyrate dehydrogenase. There were no statistically significant differences between the patient groups.

Therefore, the evidence that free radicals are formed during ischemia-reperfusion has mainly been indirect and based on determination of lipid peroxidation products. Various in vitro studies on lipid peroxidation have demonstrated that MDA reflects both autooxidation and oxygen radical–mediated peroxidation of unsaturated fatty acids.[26-29] Although MDA has been generally used as an index of lipid peroxidation, it has to be noted that MDA probably represents only a small percentage of lipid peroxides. However, the main concern has been the specificity and accuracy of the biochemical tests used to measure MDA.[30,31] The method generally used requires boiling of samples to develop a pink adduct with TBA, spectrophotometrically detectable at 535 nm. It has been suggested that the assay does not reflect actual concentrations of MDA, because the heating procedure may induce decomposition of lipid hydroperoxides and cycloperoxides. Moreover, TBA is able to form adducts with sugars, nucleic acids, and proteins, the absorbance maxima of which are very near to that of the TBA-MDA complex. Therefore, the TBA test measures "TBA-reactive substances" instead of MDA, and this is probably the reason for the conflicting results obtained in the various ischemia-reperfusion studies. In our study, MDA was determined by a recently developed HPLC method.[22] This method is much more specific than the TBA colorimetric test and avoids any additional manipulation of the plasma extracts and potential coelution of other acid-soluble compounds. In addition, the simultaneous determination of MDA and adenosine degradation products offers an opportunity to correlate peroxidative damages with the cellular energy state (estimated as net efflux of adenosine degradation products) and estimate the activity of xanthine oxidase pathway.

Our results clearly demonstrate that reperfusing the ischemic myocardium during and after aortic cross-clamping in coronary bypass surgery leads to formation of MDA and thus lipid peroxidation in the myocardium of most of the patients. This favors the hypothesis that free radical–mediated reperfusion injury also occurs in humans. The results also suggest that lipid peroxidation occurs very rapidly on reperfusion, because increased concentrations of MDA were observed in the CS blood during the short intermittent reperfusion periods as well as soon as 2 minutes after the aortic cross-clamp was released.

It was interesting to note that in two patients MDA could not be detected in any of the plasma samples taken from CS or Ao. The CS-Ao concentration differences of purine compounds in these particular patients were lower throughout the observation period. On the other hand, the MDA producers with higher transcardiac differences of purines also demonstrated higher differences of MDA. These facts suggest that myocardial energy state may be one of the determinants of lipid peroxidation and that when energy state is better preserved, the extent of lipid peroxidation is lower or can be avoided. The activities of the various antioxidant defense mechanisms may also be different among individuals.

Davies et al[10] found that in patients undergoing coronary bypass surgery, MDA and isomerized lipids increased in arterial and mixed venous blood but not in CS. On that basis, they suggested that most of the increase in these metabolites originates in tissues other than myocardium. In our study, the plasma concentrations of MDA were sixfold to ninefold lower than those measured by Davies et al,[10] and the concentrations were constantly higher in CS than in Ao. The preischemic levels were low, and MDA increased only after reperfusion favoring the idea that both ischemia and reperfusion are prerequisites for the reperfusion injury to occur. Bical et al[32] reported that the preischemic myocardial MDA levels were actually higher than those measured at the end of the aortic cross-clamp period or after 30 minutes of reperfusion. The content of MDA in left ventricular myocardium before aortic cross-clamping was 0.2 μmol/g dry wt in their study, and this is about two times higher than that we observed for rat heart muscle subjected to ischemia-reperfusion.[33,34] We believe that the discrepancies between these studies can be mainly explained by the unspecificity of the TBA colorimetric test used by Davies et al[10] and Bical et al.[32] Ferreira et al[35] showed that hydroperoxide-initiated chemiluminiscence increases on reperfusion during coronary bypass surgery, and their results are in accordance with the results obtained in our study.

Raised lipid peroxide concentrations in coronary venous blood have also been seen after myocardial ischemia induced by pacing or coronary angioplasty.[12,36] In addition, increased levels of peroxidation products in peripheral blood were observed in patients with stable and unstable angina[13] or patients with acute myocardial infarction, treated with successful fibrinolytic treatment.[37,38] Recently, Grech et al[14] were able to measure spin-trapped free radicals directly with electron paramagnetic resonance spectroscopy in patients undergoing primary coronary angioplasty for acute myocardial infarction. To our knowledge, this report represents the first direct evidence of free radical production during coronary artery recanalization in humans.

The activity of xanthine oxidoreductase is detectable in the hearts of a number of species.[18] However, there has been controversy about its activity in the human heart. Some authors have measured high activity,[19,39] whereas others report almost undetectable levels of

activity.[16,17] Huizer et al[20] measured urate production by human hearts with coronary artery disease and found that net production of urate increased in ischemia induced by balloon angioplasty. Their data are thus in accordance with ours showing that in vivo the human heart is able to produce urate and that the net production increases with ischemia. The positive correlation between the transcardiac differences in the sum of xanthine plus uric acid and MDA in our study also suggests that xanthine oxidoreductase might be involved in the generation of free oxygen radicals and lipid peroxides during coronary bypass surgery. However, because the correlation was relatively weak, other free radical–producing mechanisms (eg, arachidonate cascade, neutrofils, autooxidation of catecholamines and mitochondrial respiratory chain[7]) probably are also involved. On the other hand, the present data do not allow us to differentiate between various cellular sources of uric acid, eg, endothelial cells or working cardiomyocytes. Neutrofils, which are capable of producing large quantities of superoxide as a consequence of their NADPH oxidase activity,[40] have been suggested to play an important role in the genesis of reperfusion injury.[41-44] Small amounts of free radicals produced by xanthine oxidase could act as chemoattractants for neutrophils,[45] and thus there may a synergism between xanthine oxidase and neutrophils. As also pointed out by Huizer et al,[20] one cannot exclude direct production of urate by xanthine oxidoreductase activity of neutrophils adhered to coronary endothelium during ischemia-reperfusion.

The levels of left ventricular adenine nucleotides decrease during heart surgery with subsequent washout of diffusible degradation products.[25,46-48] On the other hand, in experimental protocols, any decrease in the myocardial energy state has been shown to correlate with increased venous concentrations of adenosine and its degradation products.[49-52] Therefore, it can be concluded on the basis of the present results that myocardial energy state decreases during the aortic cross-clamp period but then gradually recovers during the final reperfusion phase in cardiopulmonary bypass, after the heart has been revascularized (Fig 3).

Free radical–mediated lipid peroxidation has been suggested to cause postischemic myocardial dysfunction.[7,8] The two patients needing postoperative intra-aortic balloon pumping had somewhat higher transcardiac differences of MDA during aortic cross-clamping. The significance of this remains unclear, because in general more prominent lipid peroxidation was not associated with higher enzyme leakage or impaired functional recovery in this study.

In conclusion, we demonstrate that lipid peroxidation occurs in human myocardium during coronary bypass surgery. MDA is released when blood cardioplegia is delivered intermittently into the Ao and prepared grafts during aortic cross-clamp period as well as in continued cardiopulmonary bypass thereafter. Net uric acid production by myocardium occurs, showing that xanthine oxidase must also be operative in human heart. The positive correlation between the transcardiac concentration differences in MDA and xanthine plus uric acid suggests that free oxygen radicals generated in the adenine nucleotide degradation pathway are at least in part responsible for the observed lipid peroxidation. It thus appears that the myocardial energy state may be one of the determinants of the postischemic reperfusion injury. However, the level of higher lipid peroxidation, to the extent observed in this study, was not associated with poor functional recovery. More studies are needed to resolve the role of lipid peroxidation in the postischemic functional recovery of the human myocardium.

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References

1. Hearse DJ. Reperfusion of the ischemic myocardium. *J Mol Cell Cardiol.* 1977;9:605-616.
2. Braunwald E, Kloner RA. Myocardial reperfusion: a double-edged sword? *J Clin Invest.* 1985;76:1713-1719.
3. Opie LH. Reperfusion injury and its pharmacologic modification. *Circulation.* 1989;80:1049-1062.
4. Weisfeldt ML. Reperfusion and reperfusion injury. *Clin Res.* 1987; 35:13-20.
5. Kloner RA. Does reperfusion injury exist in humans? *J Am Coll Cardiol.* 1993;21:537-545.
6. Simpson PJ, Lucchesi BR. Free radicals and myocardial ischemia and reperfusion injury. *J Lab Clin Med.* 1987;110:13-30.
7. Bolli R. Oxygen-derived free radicals and postischemic myocardial dysfunction ('stunned myocardium'). *J Am Coll Cardiol.* 1988;12: 239-249.
8. Bolli R. Myocardial 'stunning' in man. *Circulation.* 1992;86: 1671-1691.
9. Ferrari R, Ceconi C, Curello S, Cargnoni A, Alfieri O, Pardini A, Marzollo P, Visioli O. Oxygen free radicals and myocardial damage: protective role of thiol-containing agents. *Am J Med.* 1991;91(suppl 3C):955-1045.
10. Davies SW, Underwood SM, Wickens DG, Feneck RO, Dormandy TL, Walesby RK. Systemic pattern of free radical generation during coronary bypass surgery. *Br Heart J.* 1990;64:236-240.
11. Bell D, Nicoll JJ, Millar A, Dawes J, Muir AL. Inflammatory response, neutrofil activation, free radical production after acute myocardial infarction: effect of thrombolytic treatment. *Br Heart J.* 1990;63:82-92.
12. Oldroyd KG, Paterson JR, Rumley AG, Eteiba H, Rae AP, Shepherd J, Cobbe SM, Hutton I. Coronary venous lipid peroxide concentrations after coronary angioplasty: correlation with biochemical and electrocardiographic evidence of ischemia. *Br Heart J.* 1992;68:43-47.
13. McMurray J, Chopra M, Abdullah I, Smith WE, Dargie HJ. Evidence for oxidative stress in unstable angina. *Br Heart J.* 1992; 68:454-456.
14. Grech ED, Dodd NJF, Bellamy CM, Perry RA, Morrison WL, Ramsdale DL. Free-radical generation during angioplasty reperfusion for acute myocardial infarction. *Lancet.* 1993;341:990-991.
15. Schoutsen B, DeJong JW, Harmsen E, De Tombe PP, Achterberg PW. Myocardial xanthine oxidase/dehydrogenase. *Biochim Biophys Acta.* 1983;726:519-524.
16. Eddy LJ, Stewart JR, Jones HP, Engerson TD, McCord JM, Downey JM. Free radical-producing enzyme, xanthine oxidase, is undetectable in human hearts. *Am J Physiol.* 1987;253:H709-H711.
17. Muxfeldt M, Schaper W. The activity of xanthine oxidase in hearts of pigs, guinea pigs, rabbits, rats and humans. *Basic Res Cardiol.* 1987;82:486-492.
18. Downey JM, Hearse DJ, Yellon DM. The role of xanthine oxidase during myocardial ischemia in several species including man. *J Mol Cell Cardiol.* 1988;20:55-63.
19. Wajner M, Harkness RA. Distribution of xanthine dehydrogenase and oxidase activities in human and rabbit tissues. *Biochem Soc Trans.* 1988;16:358-359.
20. Huizer T, DeJong JW, Nelson JA, Czarnecki W, Serruys PW, Bonnier JRM, Troquay R. Urate production by human heart. *J Mol Cell Cardiol.* 1989;21:691-695.
21. Thompson-Gorman SL. Evaluation of the role of xanthine oxidase in myocardial reperfusion injury. *J Biol Chem.* 1990;265:6656-6663.
22. Lazzarino G, Di Pierro D, Tavazzi B, Cerroni L, Giardina B. Simultaneous separation of malondialdehyde, ascorbic acid, and adenine nucleotide derivatives from biological samples by ion-pairing high-performance liquid chromatography. *Anal Biochem.* 1991;197:191-196.

23. Möser GH, Schrader J, Deussen A. Turnover of adenosine in plasma of human and dog blood. *Am J Physiol.* 1989;307:404-409.
24. Peuhkurinen K, Ikäheimo M, Airaksinen J, Huikuri H, Linnaluoto M, Takkunen J. Changes in myocardial energy metabolism in elective coronary angioplasty. *Cardiovasc Res.* 1991;25:158-163.
25. Nissinen J, Raatikainen MJP, Karlqvist K, Peuhkurinen KJ. Efflux of adenosine and its catabolites during cold blood cardioplegia. *Ann Thorac Surg.* 1993;55:1546-1552.
26. Braughler JM, Duncan LA, Chase RL. The involvement of iron in lipid peroxidation (importance of ferric to ferrous ratios in initiation). *J Biol Chem.* 1986;261:10282-10289.
27. Kostrucha J, Kappus H. Inverse relationship of ethane or N-pentane and malondialdehyde found during lipid peroxidation in rat liver microsomes with different oxygen concentrations. *Biochim Biophys Acta.* 1986;879:120-125.
28. Frankel EN. Lipid oxidation: mechanisms, products and biological significance. *J Am Chem Soc.* 1984;61:1908-1916.
29. Janero DR, Burghardt B. Thiobarbituric acid-reactive malondialdehyde formation during superoxide-dependent, iron-catalyzed peroxidation: influence of peroxidation conditions. *Lipids.* 1989;24: 125-131.
30. Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clin Chim Acta.* 1978; 90:37-43.
31. Gutteridge JMC. Free radical damage to lipids, amino acids, carbohydrates and nucleic acids determined by thiobarbituric acid reactivity. *Int J Biochem.* 1982;14:649-653.
32. Bical O, Gerhardt MF, Paumier D, Gaillard D, Comas J, Landais P, Fischer M, Trivin F, Vanetti A. Comparison of different types of cardioplegia and reperfusion on myocardial metabolism and free radical activity. *Circulation.* 1991;84(suppl III):III-375-III-379.
33. Tavazzi B, Lazzarino G, Di Pierro D, Giardina B. Malondialdehyde production and ascorbate decrease are associated to the reperfusion of the isolated post-ischemic rat heart. *Free Rad Biol Med.* 1992;13:75-79.
34. Di Pierro D, Tavazzi B, Lazzarino G, Giardina B. Malondialdehyde is a biochemical marker of peroxidative damage in the isolated reperfused rat heart. *Mol Cell Biochem.* 1992;116:193-196.
35. Ferreira R, Liesuy S, Milei J, Scordo D, Hourquebie H, Molteni L, de Palma C, Boveris A. Assessment of myocardial oxidative stress in patients after myocardial revascularization. *Am Heart J.* 1988; 115:307-312.
36. Oldroyd KG, Chopra M, Rankin AC, Belch JJF, Cobbe SM. Lipid peroxidation during myocardial ischemia induced by pacing. *Br Heart J.* 1990;63:88-92.
37. Davies SV, Randajadayalan K, Wickens DG, Dormandy TL, Timmis AD. Lipid peroxidation associated with successful thrombolysis. *Lancet.* 1990;335:741-743.
38. Giardina B, Penco M, Lazzarino G, Romano S, Tavazzi B, Fedele F, Di Pierro D, Dagianti A. Effectiveness of thrombolysis is associated with a time-dependent increase of malondialdehyde in peripheral blood of patients with acute myocardial infarction. *Am J Cardiol.* 1993;71:788-793.
39. Krenitsky TA, Tuttle JV, Cattau EL, Wang P. A comparison of the distribution and electron acceptor specificities of xanthine oxidase and aldehyde oxidase. *Comput Biochem Physiol.* 1974;49B:687-703.
40. Weining RS, Wever R, Roog D. Quantitative aspects of the superoxide radical phagocytosing human granulocytes. *J Lab Clin Med.* 1975;85:245-252.
41. Romson JL, Hook BG, Kunkel SL, Abrams GD, Schork MA, Lucchesi BR. Reduction of the extent of ischemic myocardial injury by neutrofil depletion in the dog. *Circulation.* 1983;67: 1016-1023.
42. Rowe GT, Manson NH, Caplan M, Hess ML. Hydrogen peroxide and hydroxyl radical mediators of activated leukocyte depression of cardiac sarcoplasmic reticulum. *Circ Res.* 1983;53:584-591.
43. Menasche P, Pasquier C, Bellucci S, Lorente P, Jaillon P, Piwnica A. Deferoxamine reduces neutrofil-mediated free radical production during cardiopulmonary bypass in man. *J Thorac Cardiovasc Surg.* 1988;96:582-589.
44. Engler RL. Free radical and granulocyte-mediated injury during myocardial ischemia and reperfusion. *Am J Cardiol.* 1989;63: 19E-23E.
45. McCord JM. The superoxide free radical: its biochemistry and pathophysiology. *Surgery.* 1983;94:412-414.
46. Kaijser L, Jansson E, Schmidt W, Bomfim V. Myocardial energy depletion during profound hypothermic cardioplegia for cardiac operations. *J Thorac Cardiovasc Surg.* 1985;90:896-900.
47. Sollevi A, Schmidt W, Jansson E, Bomfim V, Kaijser L. Adenine nucleotide degradation in human myocardium during cardioplegia. *Cardiovasc Res.* 1987;21:358-361.
48. Weisel RD, Mickle DAG, Finkle CD, Tumiati LC, Madonik MM, Ivanov J. Delayed myocardial metabolic recovery after blood cardioplegia. *Ann Thorac Surg.* 1989;48:503-507.
49. Manfredi JP, Sparks HV. Adenosine's role in coronary vasodilatation induced by atrial pacing and norepinephrine. *Am J Physiol.* 1982;243:H536-H545.
50. Kiviluoma KT, Peuhkurinen KJ, Hassinen IE. Role of cellular energy state and adenosine in the regulation of coronary flow during variation in contraction frequency in an isolated perfused rat heart. *J Mol Cell Cardiol.* 1986;18:1133-1142.
51. Raatikainen MJP, Peuhkurinen KJ, Hassinen IE. Cellular source and role of adenosine in isoproterenol-induced coronary vasodilatation. *J Mol Cell Cardiol.* 1991;23:1137-1148.
52. Uusimaa PA, Peuhkurinen KJ, Vuolteenaho O, Ruskoaho H, Hassinen IE. Role of myocardial redox and energy states in ischemia stimulated release of atrial natriuretic peptide. *J Mol Cell Cardiol.* 1992;24:191-205.