ORIGINAL ARTICLES

Systemic pattern of free radical generation during coronary bypass surgery

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Abstract

Diffuse impairment of ventricular function after cardiac surgery may be related to the generation during reperfusion of the myocardium of free radicals derived from oxygen. Fifteen patients undergoing elective coronary bypass surgery were studied by previously described assays for peroxidised lipids and for isomerised lipids which were used as indices of free radical activity. Serial blood samples were obtained from systemic arterial, mixed venous, and coronary sinus catheters before, during, and after the ischaemic period. The patients underwent coronary artery surgery on cardiopulmonary bypass with a membrane oxygenator, relative hypothermia 30–34°C, and intermittent cross-clamping of the aorta. During the ischaemic periods there were no significant changes in the indices of free radical activity. During the reperfusion phase there was a significant increase in free radical indices in arterial and mixed venous blood. A small rise in free radical indices in coronary venous blood was not statistically significant.

These data indicate that free radical activity is increased in patients shortly after the cessation of cardiopulmonary bypass. The pattern of distribution between the different sampling sites suggests that much of the observed increase in isomerised and peroxidised lipids originates from tissues other than the myocardium.

During coronary artery surgery the heart undergoes periods of ischaemia with either cardioplegia or aortic cross-clamping and induced ventricular fibrillation. There is sometimes a significant global deterioration in left ventricular function that is attributed to this ischaemia and to the subsequent reperfusion.^{1,2} Reperfusion is thought to generate free radicals derived from oxygen when oxygen is reintroduced during reperfusion of the myocardium.^{3–5} Free radicals are chemical species with an unpaired electron and are highly reactive. Free radicals derived from oxygen can damage a wide variety of biological molecules including proteins, lipids, and nucleic acids.⁶ This will impair cell structure and function; in particular, damage to

membrane lipids may allow the influx of ionised calcium.^{6–8} It has been suggested that changes in the cell membrane are also responsible for reperfusion arrhythmias.⁹

Diffuse pulmonary impairment can also occur after bypass surgery; this is similar both histologically and functionally to the adult respiratory distress syndrome associated with severe systemic infection or major trauma.¹⁰ During cardiopulmonary bypass neutrophils were sequestered in the lungs,^{11,12} with the release of enzymes and the generation of lipids damaged by free radicals.^{11,13,14}

Both the diffuse impairment of ventricular function and the pulmonary impairment after bypass surgery may therefore be related to the generation of free radicals derived from oxygen. To investigate this we used two independent measures of free radical damage to study patients during bypass surgery.

Patients and methods

PATIENT SELECTION

Fifteen patients undergoing elective coronary artery bypass grafting gave informed consent for the placement of coronary sinus and pulmonary arterial catheters and for blood sampling during the bypass procedure. All were men (aged 41 to 67 years (mean age 54)). Patients had good left ventricular function as judged by the preoperative angiogram (left ventricular ejection fraction >55%) and had no evidence of pulmonary disease as judged by chest x ray, lung volumes, and carbon monoxide gas transfer (diffusing capacity).

PROCEDURE

After premedication a Baim-Rothman catheter was inserted into the coronary sinus and its position confirmed (a) by obtaining blood samples with oxygen saturation <45% and (b) by radiography during the injection of small volumes of non-ionic contrast medium. A Swan-Ganz catheter was inserted into the central pulmonary artery and a radial artery cannula was inserted.

Anaesthesia was induced with a benzodiazepine/narcotic/relaxant sequence and the patient was ventilated with 50% oxygen in nitrogen. Anaesthesia was maintained with further intravenous or inhalational anaesthetics, as required. Patients were perfused during cardiopulmonary bypass with non-pulsatile flow from a sheet membrane oxygenator (COBE membrane lung) with a 2 litre crystalloid prime. The surgical technique con-

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Figure 1 Plasma concentrations (mean (SEM)) of malondialdehyde during cardiopulmonary bypass, measured as thiobarbituric acid reacting material.

sisted of sequential myocardial revascularisation by vein and internal mammary artery grafts with intermittent cross-clamping of the aorta, ventricular fibrillation, and mild hypothermia 32–34°C.

Serial sets of blood samples were drawn from the mixed venous site (central pulmonary artery during bypass from the venous line to the pump), systemic arterial site (radial artery cannula), and coronary sinus (Baim-Rothman catheter). Blood was drawn into heparinised plastic syringes, and the plasma was separated by centrifugation and stored in plastic tubes at –20°C. Samples were taken (a) after induction of anaesthesia; (b) after systemic heparinisation, on cardiopulmonary bypass; (c) during the last of the periods of cardiac ischaemia (aortic cross-clamping with fibrillation); and 2, 5, and 10 minutes after coronary reperfusion.

LIPID PEROXIDATION ASSAY

Material that reacted with thiobarbituric acid was measured according to a modification of the fluorimetric method of Yagi.^{15,16} This is preferred to the spectrophotometric assay, in which bilirubin, glucose, and sialic acid may interfere. Volumes in the procedure differed from Yagi's original method in two respects: plasma samples of 50 µl rather than 20 µl were used, and adduct was extracted into 3 ml butan-

Figure 2 Molar ratios (mean (SEM)) of 18:2(9,11)/18:2(9,12) in plasma free fatty acids during cardiopulmonary bypass.

Figure 3 Molar ratios (mean (SEM)) of 18:2(9,11)/18:2(9,12) in plasma phospholipids during cardiopulmonary bypass.

1-ol rather than 5 ml. This increases the sensitivity and improves the repeatability of the assay. To take into account haemodilution during bypass the plasma albumin was determined on a Beckman Astra 8 with a bromocresol green reagent, and results were expressed as grams thiobarbituric acid reactive material per gram albumin.

LIPID ISOMERISATION ASSAY

In vitro experiments showed that free radicals can cause isomerisation of polyunsaturated fatty acyl chains without causing their oxidation.¹⁷ The isomerisation most studied has been the conversion of linoleic acid (18:2(9,12)) to octadeca-9,11-dienoic acid (18:2(9,11)), which accounts for over 90% of diene conjugation in human plasma, tissues, and tissue fluids.¹⁸ A high performance liquid chromatographic method can measure both the isomer 18:2(9,11) and the parent 18:2(9,12) in plasma.¹⁹ Plasma samples were separated into free fatty acid and phospholipid fractions, and these were analysed separately. To take into account haemodilution during bypass, the concentration of 18:2(9,11) was expressed as the molar ratio of isomerised product to parent molecule—that is, $18:2(9,11) \times 100\% / 18:2(9,12)$.

STATISTICAL ANALYSIS

Baseline values of the indices of free radical activity varied widely and because a normal distribution could not be assumed we used non-parametric methods. We used the Wilcoxon matched pairs rank test to make comparisons between different points in time. We adjusted significance values for multiple comparisons using the Bonferroni correction.

Results

The anaesthetic, bypass, and surgical procedures were uncomplicated in all patients and between 1 and 4 grafts were performed in each patient. All patients had an uncomplicated postoperative course without clinical signs of

impaired ventricular function with normal echocardiographic appearances and no new changes in the 12 lead electrocardiogram.

Initial values of all the free radical indices varied widely. Concentrations of material reacting with thiobarbituric acid ranged from 66 to 145 ng per gram of albumin. The molar ratio 18:2(9,11)/18:2(9,12) in the phospholipid fraction ranged from 1.45% to 7.57% and the molar ratio in the free fatty acid fraction ranged from 1.92% to 10.36%.

During the period of cardiac ischaemia there were no significant changes in either concentrations of material reacting with thiobarbituric acid or in the molar ratio at any of the sites of sampling. During the reperfusion phase, however, there were several significant changes. The concentration of material reacting with thiobarbituric acid increased significantly in arterial blood ($p < 0.01$) and in mixed venous blood ($p < 0.01$) but increases in coronary sinus blood were smaller and not significant (fig 1). The molar ratio 18:2(9,11)/18:2(9,12) in the free fatty acid fraction increased slightly after systemic heparinisation and the start of cardiopulmonary bypass (fig 2). At the time of reperfusion this molar ratio increased significantly in mixed venous blood ($p < 0.01$) and in arterial blood ($p < 0.05$); a small increase in the coronary sinus blood was not significant (fig 2). The molar ratio in the phospholipid fraction increased significantly in mixed venous ($p < 0.01$) and in arterial blood ($p < 0.05$), but again there were no significant changes in coronary sinus blood (fig 3).

Discussion

Protection of the myocardium so as to minimise the impairment of left ventricular function is a major concern during cardiac surgery. Left ventricular function is an independent determinant of prognosis in various cardiac conditions.²⁰⁻²² It has been postulated that reperfusion injury makes a significant contribution to postoperative ventricular dysfunction²³; this is of considerable practical importance in influencing strategies to improve myocardial preservation.

The central role of free radicals derived from oxygen in diverse forms of cardiac disease is increasingly appreciated.²⁴⁻²⁵ They have been implicated in anthracycline cardiotoxicity,²⁶⁻²⁷ the cardiomyopathy of thalassaemia and haemochromatosis,²⁸⁻²⁹ and in selenium deficiency cardiomyopathy.³⁰⁻³¹ Most work, however, relates to the phenomenon of reperfusion injury and the implications for cardiac surgery, thrombolysis, and angioplasty.⁴⁻⁵ Because the free radicals themselves are highly reactive, with life spans of the order of microseconds or shorter, they can only be measured directly in tissues and in cell-free systems by electron spin resonance spectroscopy (electron paramagnetic resonance). The direct detection of free radicals in cardiac cells and tissues has so far been possible only in vitro and in animal experiments.³²⁻³³

In most studies, free radical activity has been inferred indirectly by measurement of bio-

logical molecules that have been damaged so as to produce unusual products. There are assays for damaged proteins and peptides,³⁴ damaged nucleic acids,³⁵ and damaged lipids.³⁶ Lipid damage has been studied in particular detail, and it is known that oxygen free radicals can initiate a self-propagating chain reaction of lipid peroxidation³⁷; thus in published studies, the most commonly used indices of free radical activity have been based on lipid peroxidation. Best known is the assay of malondialdehyde that uses the reagent thiobarbituric acid; there are, however, some limitations in the use of this assay. Although material reactive to thiobarbituric acid is mainly derived from cyclic endoperoxides of lipids that are the result of free radical damage, it is not entirely specific for these.³⁸⁻³⁹ Furthermore, some common biological substances may interfere with the spectrophotometric measurement of such material.⁴⁰

Also not all free radical damage to lipids results in peroxidation, and so will not be detected by an assay for malondialdehyde. In vitro experiments showed that free radicals can cause isomerisation of polyunsaturated fatty acyl chains, without causing their oxidation.¹⁷ It therefore seems that there are two possible pathways of lipid damage after free radical interaction (fig 4) and thus an index of each pathway is necessary to assess fully the extent of lipid damage. The lipid isomerisation most studied has been the conversion of linoleic acid (18:2(9,12)) to octadeca-9,11-dienoic acid (18:2(9,11)),¹⁸⁻³⁶ and this formed the basis of one of the assays of free radical activity in the present study.

Despite the problems of these indirect measurements they have been widely used in in vitro models and in animal experiments, and suggest considerable generation of free radicals at the time of reperfusion, synchronous with a further decline in myocardial contractile function. Only recently have such measurements been attempted in humans—after surgical revascularisation of the myocardium and during angina induced by pacing.⁴¹⁻⁴³

It is possible that the generation of free radicals derived from oxygen is an epiphenomenon and not related to reperfusion damage. In several experimental models, however, there are parallel changes in the degree of cell damage and the level of free radical generation, suggesting a causative role. If perfusion is restored to the ischaemic zone gradually rather than suddenly, both the level of free radical generation and the extent of structural and functional damage are much reduced.⁴⁴ Similarly if perfusion is restored with oxygen-free fluid equilibrated with 100% nitrogen, and oxygen is then gradually reintroduced, there is a similar limitation both of free radical generation and of damage.⁴⁵

Various drugs are thought to protect against damage by free radicals derived from oxygen, either by limiting their generation or by "scavenging" them. These include enzymes (superoxide dismutase, catalase), molecules with thiol groups (glutathione, captopril), mannitol, and chelators of transition metal ions

Figure 4 Postulated pathways of lipid damage by free radicals.

(desferrioxamine). In systems in vitro and in animal experiments these drugs both limit the generation of free radicals, as judged by electron spin resonance spectroscopy and by lipid markers, and reduce structural damage, impairment of contraction, and arrhythmias on reperfusion of ischaemic myocardium.^{32 46-48} The single common feature of these chemically diverse drugs is that they reduce the impact of free radicals derived from oxygen, and thus the above observations strongly suggest that free radicals play a causal role in reperfusion injury. There has been particular interest in the possible benefits of adding these agents to cardioplegic solutions, but despite the large body of experimental evidence, drugs for scavenging free radicals have yet to be evaluated in clinical practice.

Finally the administration of chemical systems that generate free radicals has been shown to be injurious, both in vitro and in animal models.³² The generation of free radicals in situ by photochemical reaction also causes severe functional and structural changes resembling reperfusion injury.^{49 50} Clearly the corresponding experiments in humans are impossible.

Permanent global impairment of left ventricular function is an important though fortunately uncommon complication of coronary artery surgery with cardiopulmonary bypass.¹² It has been suggested that the permanent damage arises at the time of reperfusion, as opposed to the temporary depression of function observed during the period of ischaemia itself.^{3 4} A series of ventricular biopsy specimens taken from six patients undergoing operative correction of tetralogy of Fallot showed diene conjugation, a marker of free radical activity, coinciding with myocyte necrosis.⁵¹ In the present study, however, we found no evidence of increased free radical activity in samples of coronary sinus blood at the time of reperfusion. Indeed, there were no significant changes in either of the indices of free radical damage to lipids in coronary sinus blood, during either the ischaemic period of fibrillation and cross-clamping, or at 2, 5, and

10 minutes after coronary reperfusion. In contrast, there were significant increases in these indices in the systemic blood samples; this suggests that much of the observed rise originated from tissues other than the myocardium.

Similar increases in the molar ratio in free fatty acid in venous blood were seen in normal individuals after the administration of 5 000 units heparin,⁵² and the increased molar ratio in free fatty acid in the present study may simply reflect heparinisation. Heparin did not cause changes in the molar ratio in phospholipid,⁵² and thus cannot account for the increase after bypass. The increase in material reactive to thiobarbituric acid was proportionally greater than the increases in the molar ratio and in the absolute concentration of 18:2(9,11). The isomer 18:2(9,11) is not a peroxidation product and might be expected to increase under anoxic, ischaemic conditions rather than on reperfusion and reoxygenation.

None of the 15 patients in the present study suffered any measurable impairment of left ventricular function, so we do not know whether or not severe perioperative left ventricular impairment would have been associated with an increase in free radical markers in the coronary sinus blood. Certainly in these patients with uncomplicated procedures the increase in free radical markers was *least* in the coronary sinus samples, and did not represent a significant change from the baseline value.

The site of origin of these lipids damaged by free radicals is not defined by the present study, but other work suggests that the lungs are a major source. At the time of restoration of the circulation and of perfusion to the heart, perfusion to the lungs is also restored and there is evidence of white cell sequestration within the lungs.^{11 12} Activated components of the complement system, markers of white cell degranulation, and markers of free radical activity were all detectable in the pulmonary effluent blood at this time.^{11 13 14} Though few studies have attempted directly to link these changes with functional or structural impairment, they may well underlie the diffuse pulmonary im-

pairment that is common after bypass and which sometimes causes the clinically important syndrome resembling adult respiratory distress syndrome.¹⁰

CONCLUSIONS

The increase in both peroxidised and isomerised lipids in the systemic circulation during the first few minutes of reperfusion was consistent with free radical damage at this time. This rise was least in blood from the coronary sinus, suggesting that much of the rise originated from tissues other than the myocardium. Slightly different patterns of change in lipids damaged by free radicals were seen, depending on which index of free radical damage was used.

We recommend that further studies should (a) specifically sample the coronary sinus as well as systemic sites; (b) use more than one index of free radical activity to assess reliably the total extent of lipid damage via the different pathways. The methods and problems of the assays of free radical markers have been fully described by others.^{18,40} Because other factors may interfere with the assays diet and drug treatment must be monitored.

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