Evidence for oxidative stress in unstable angina

John McMurray, Mridula Chopra, Ibrahim Abdullah, W Ewen Smith, Henry J Dargie

Abstract

Objective—To determine whether unstable angina, which is characterised by recurring episodes of myocardial ischaemia and reperfusion, is associated with oxidative stress (that is, where there is an imbalance between oxidants, such as free radicals, which are in excess and antioxidants).

Design—Between group comparison of patients with unstable angina, stable angina, and healthy controls.

Setting—The coronary care unit and cardiac investigation ward of a regional cardiology centre.

Patients—Twenty five consecutive patients admitted to the coronary care unit with unstable angina. Twenty five consecutive patients admitted to the cardiac investigation ward (patients with stable angina undergoing coronary angiography) were used as controls for the presence of atherosclerosis, drug treatment, and smoking habit. Thirty eight healthy controls (hospital staff and patients admitted for minor surgical procedures who were otherwise healthy) were also studied.

Main outcome

measures—Thiobarbituric acid related substances (TBARS) in plasma and plasma reduced thiol (PSH) as indicators of oxidative damage to lipids and proteins respectively were measured. Coronary angiography was performed in all patients with stable angina and roughly half of those with unstable angina.

Results—Mean (SEM) plasma TBARS in unstable angina and stable angina were 9.95 (0.36) nmol/ml and 9.14 (0.28) nmol/ml respectively ($p = 0.08$). Mean plasma TBARS in healthy controls were 8.09 (0.21) nmol/ml ($p < 0.05$ compared with both angina groups). Mean (SEM) PSH concentration in unstable angina was 4.21 (9) nmol/ml and in stable angina was 4.85 (9) nmol/ml ($p < 0.05$). Mean PSH in healthy controls was 5.64 (8) nmol/ml ($p < 0.001$ compared with both angina groups). The extent of coronary artery disease, use of medication, and smoking habit were not significantly different between the angina groups.

Conclusions—Biochemical indicators of oxidative stress are more abnormal in unstable than stable angina. This is in keeping with experimental evidence that episodes of ischaemia and reperfusion lead to generation of free radicals and

toxic oxygen species and depression of endogenous antioxidant activity. The clinical significance of this finding remains to be determined, although, experimentally, free radicals and toxic oxygen species have adverse effects on myocardial contractile function, myocardial electrical stability, endothelial mediated vasodilatation, and coagulation.

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Free radicals and related toxic oxygen species have been implicated in a growing number of disease processes. Two mechanisms common to these processes are thought to lead to generation of free radicals and toxic oxygen species: these are the sequence of tissue ischaemia followed by reperfusion, and the activation of neutrophil nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and myeloperoxidase in the respiratory burst.¹⁻³ Endogenous antioxidant defences may also be depressed in these circumstances.¹⁻³ Where there is an imbalance between oxidant (eg, free radical) and antioxidant activity in favour of the oxidant oxidative stress is said to exist. Free radicals and associated toxic oxygen species are highly unstable chemically and immediately react with adjacent biomolecules to cause cellular and tissue damage.¹⁻³ In experimental models contractile and vascular endothelial injury can be caused in the heart by free radicals and toxic oxygen species.³⁻⁵ Clinically, one common condition characterised by recurring episodes of myocardial ischaemia and reperfusion and neutrophil activation is unstable angina.⁶ We have sought biochemical evidence of oxidative stress in patients with unstable angina. Concentrations of indicators of free radical damage to lipids (lipid peroxidation—that is, plasma thiobarbituric acid reactive substances (TBARS)) and proteins (thiol or sulphhydryl group oxidation—that is, decreased plasma reduced thiol (PSH)) were measured.^{1,7} Where there is an imbalance between oxidants (for example, free radicals) and antioxidants in favour of oxidants (where there is oxidative stress), lipid peroxidation is increased and protein thiols are decreased.^{1,7}

Patients and methods

PATIENTS

Unstable angina

Twenty five consecutive patients admitted to the coronary care unit with a confirmed diagnosis of unstable angina were studied. Unstable angina was defined as an increase in frequency or severity or both of existing angina or recent

Department of
Cardiology, Western
Infirmary, Glasgow

J McMurray
I Abdullah
H J Dargie

Department of
Chemistry, University
of Strathclyde,
Glasgow

M Chopra
W E Smith

Correspondence to:
Dr John McMurray,
Department of Cardiology,
Western Infirmary, Glasgow
G11 6NT.

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onset angina requiring a stay in hospital. ST segment depression or T wave inversion had to be present on the electrocardiogram, and patients who had a rise in creatine kinase > 150 iu/l within 24 hours of admission were excluded from analysis. Patients with postinfarct angina were not recruited.

Stable angina

Twenty five consecutive patients with chronic stable angina and a positive exercise tolerance test (that is one associated with a > 1 mm ST segment depression by the end of stage III of the Bruce protocol) were recruited. All of these patients had been admitted for elective cardiac catheterisation under the care of one consultant cardiologist.

This study was approved by the West Ethical Committee of the Greater Glasgow Health Board and all patients gave informed consent before the study.

MEDICATION AND SMOKING HABIT

Oral and parenteral cardiac medication prescribed at the time of study were recorded. Smoking habit was also recorded.

CARDIAC CATHETERISATION

Patients with unstable angina were catheterised before discharge if their symptoms failed to settle on medical treatment, or if after discharge they had disabling angina despite medical treatment, or a positive exercise tolerance test. The coronary angiograms were reported independently of the biochemical analyses. A stenosis of $> 50\%$ was taken as significant.

NORMAL CONTROLS

Healthy controls were recruited from the hospital staff and from patients admitted for minor surgical procedures.

BLOOD SAMPLES

All blood samples were taken at roughly 08.30 hours, after an overnight fast. The samples were taken from the patients with unstable angina the first morning after admission, and from the stable angina patients the morning after admission but before catheterisation. Samples were taken from an antecubital vein into chilled lithium heparin tubes and centrifuged immediately at 5.0°C . Plasma was separated and analysed the same day.

BIOCHEMICAL ASSAYS

Plasma concentrations of TBARS (malondialdehyde, MDA) were measured as already described.⁸ Spectrophotometrical measurements of PSH were made with Ellmans reagent. The within and between assay coefficients of variance for these assays were: malondialdehyde 5% within and 6% between assays; PSH 2% within and 3% between assays.

STATISTICAL ANALYSIS

An unpaired *t* test was used to compare the three groups with Bonferroni's correction for multiple comparisons.

Results

CLINICAL CHARACTERISTICS

Healthy controls

Thirty eight controls (16 men) aged 21 to 65 years were recruited. None was on regular medication. Ten were current smokers.

Unstable angina

Patients aged 32 to 80 (mean 57 (SEM 2)) years, 15 men and 10 women were recruited. Nine were current smokers. In 80% treatment was with a β blocker, 76% with a nitrate, and 44% with a calcium channel blocker. Aspirin was taken by 84% and heparin by 28%. One patient received streptokinase and one patient had ventricular fibrillation (neither had had an infarction). One patient had a myocardial infarction more than 24 hours after admission. Twelve patients had a past myocardial infarction. Thirteen patients (52%) underwent cardiac catheterisation. Single vessel disease was present in 23% double vessel disease in 15%, and triple vessel disease in 46%; four (16%) patients had had coronary artery bypass grafting.

Stable angina

Results were analysed for 20 men and four women (one woman patient had normal coronary arteries) aged 38–70 (mean (SEM) 58 (2)) years. Twelve patients were current smokers. The treatment for 71% was with a β blocker, 67% with a nitrate, 71% with a calcium channel blocker, and 42% were taking aspirin. Five patients had a past myocardial infarction. Twenty nine per cent of patients had single vessel disease, 13% double vessel disease, and 42% triple vessel disease; four (17%) had had coronary artery bypass grafting.

Figure 1 Plasma concentrations of thiobarbituric acid related substances (malondialdehyde (MDA)) in healthy controls (normal), stable angina patients, and patients with unstable angina. Individual values are given with mean values indicated by a horizontal bar. *, $p < 0.02$ between angina groups and normal. Bracket denotes statistical comparison of the two angina groups.

Figure 2 Plasma thiol concentration (PSH) in each of the three groups as shown in fig 1. **, $p < 0.01$ for comparisons as in fig 1.

BIOCHEMICAL MEASURES OF OXIDATIVE STRESS TBARS (malondialdehyde)

Both groups of angina patients had significantly higher plasma malondialdehyde concentrations than the normal controls (fig 1). Mean plasma malondialdehyde seemed to be higher in patients with unstable angina compared with those with stable angina although this difference did not reach significance ($p = 0.08$).

PSH

In both groups of angina patients compared with controls PSH was significantly lower (fig 2), and PSH was also significantly lower in patients with unstable angina compared with those with stable angina (fig 2).

Discussion

Both groups of patients with coronary artery disease had abnormal indicators of oxidative stress in keeping with previous findings.^{9,10} Interestingly, however, this abnormality was greatest in those patients with symptomatically overt ischaemia and could not be explained by such differences as extent of coronary disease or medication. This is in keeping with the belief that ischaemia and reperfusion, or neutrophil activation, or both cause generation of free radicals and other oxidants. Similar abnormalities in both markers measured in this study were noted in another study of myocardial ischaemia and reperfusion. In that study, Davies *et al* noted a rise in TBARS and a fall in PSH in patients with myocardial infarction who successfully reperfused after thrombolytic treatment.^{11,12}

In our study the concentrations of the biochemical measures showed considerable overlap. This may reflect the fact that even stable angina patients have frequent episodes of recurring ischaemia (symptomatic or silent) and this may reduce the biochemical differences between the two angina groups. Decreased PSH has also been found in patients with stable angina and acute myocardial infarction.^{9,13} Similarly, TBARS are increased after pacing induced ischaemia and during percutaneous transluminal coronary angioplasty in humans.^{14,15}

What might the relevance of these findings be? It is known that abnormalities of wall motion may persist for days to weeks after an episode of unstable angina.¹⁶ Such dysfunction seems to be the clinical correlate of the reversible experimental mechanical reperfusion injury that is apparently caused by the generation of free radicals and oxidants.¹⁶ Oxidative stress may also provoke the arrhythmias that occur in a few patients with unstable angina (ventricular fibrillation occurred in one patient in this study).^{4,5} Lipid peroxides, products of free radical damage to lipids, may cause damage to endothelial cells, decrease generation of vascular prostacyclin, and promote thrombosis.^{5,9,10} All of these effects are potentially relevant and disadvantageous in patients with unstable angina.

LIMITATIONS

There are several limitations to this study. Methods of measuring lipid peroxides using thiobarbituric acid have been criticised on the basis of specificity. Nevertheless, this type of test remains an extremely sensitive one and is the most commonly used biochemical measure of oxidative stress. Abnormalities detected by us with the particular method used in this study have previously correlated well with other measures of redox state such as glutathione and superoxide dismutase;¹⁷ also another marker, PSH, was used in this study to corroborate free radical damage.

A second limitation is that only a proportion of patients with unstable angina underwent cardiac catheterisation. It may therefore be argued that the extent of coronary disease in the whole unstable group could be greater than in the stable group. This would mean, however, that those in the unstable group who were not catheterised would have to have had more extensive coronary disease than those who were catheterised. This is highly unlikely as in our unit catheterisation is performed on the basis of either (a) failure to respond symptomatically to treatment or (b) the finding of a positive exercise tolerance test on medical treatment. It is probable, therefore, that those in the unstable group who were not catheterised had less rather than more extensive coronary disease. Thus it seems unlikely that the addition of non-catheterised unstable patients could have imbalanced the groups in terms of extent of coronary artery disease, in a way that would invalidate the results.

In summary we have shown that unstable angina is associated with abnormal plasma biochemical markers of oxidative stress. These findings support several recent findings in patients with other forms of myocardial ischaemia and reperfusion. The clinical relevance of these findings cannot be determined from this preliminary study. Unstable angina is, however, often associated with mechanical dysfunction and thrombotic coronary occlusion, both potential consequences of the generation of free radicals and toxic oxygen species. An association between abnormally high concentrations of indicators of oxidative stress and ventricular dysfunction or the occurrence of myocardial infarction would strengthen the case that biological oxidants including free radicals are clinically important.

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