Coronary venous lipid peroxide concentrations after coronary angioplasty: correlation with biochemical and electrocardiographic evidence of myocardial ischaemia

K G Oldroyd, J R Paterson, A G Rumley, H Eteiba, A P Rae, J Shepherd, S M Cobbe, I Hutton

Abstract

**Background**—Raised lipid peroxide concentrations in coronary venous plasma have been reported after coronary angioplasty in humans. This may reflect increased free radical activity after myocardial ischaemia and reperfusion. If so, it may be possible to correlate lipid peroxide concentrations with the degree of myocardial ischaemia produced during angioplasty.

**Methods**—15 patients (age range 42–70; 12 men) with stable angina pectoris undergoing angioplasty of a proximal left anterior descending coronary artery stenosis were studied. Plasma lipid peroxide and lactate concentrations were measured in sequential blood samples taken from the great cardiac vein before and immediately after one to five serial 60 second balloon inflations. The maximum ST segment shift during each balloon inflation was also measured.

**Results**—Lipid peroxide concentrations in coronary venous plasma were raised from pre-angioplasty values by more than 2 SDs of the relevant measurement error after 27 out of 46 (59%) balloon inflations. Lactate concentrations were raised after 43 out of 46 (93%) balloon inflations. No significant difference was found between the peak percentage change of either lipid peroxide or lactate concentrations after any of the first three serial inflations. The maximum ST segment shift after each of the first three serial inflations was also similar. Coronary venous lactate concentrations after balloon inflation correlated positively with the maximum ST segment shift, but did not correlate with lipid peroxide concentrations.

**Conclusions**—Raised lipid peroxide concentrations in coronary venous plasma can be detected in humans after balloon angioplasty. There is no positive correlation between lipid peroxide concentrations in coronary venous plasma after angioplasty and the degree of preceding myocardial ischaemia as assessed by either ST segment shift or lactate production. These indices showed that one to three serial 60 second balloon inflations each produce a similar degree of myocardial ischaemia. The origin of the raised lipid peroxide concentrations in coronary venous plasma after angioplasty remains unknown.

Oxygen derived free radicals are produced during reperfusion of the ischaemic myocardium. Such free radical activity is difficult to measure directly in humans, but can result in oxidative damage to polyunsaturated membrane phospholipids with the production of relatively stable lipid peroxides. Plasma lipid peroxides are most commonly measured by catalysing their degradation to malondialdehyde, which reacts with thiobarbituric acid to form an adduct. Thus lipid peroxide concentration is normally expressed as uM of malondialdehyde and includes any free malondialdehyde that is also present in plasma. We have previously identified raised lipid peroxide concentrations in coronary venous blood after myocardial ischaemia induced by pacing. During percutaneous transluminal coronary angioplasty, recurrent brief periods of myocardial ischaemia are followed by reperfusion, and this sequence might be expected to provoke free radical generation. In an initial study of patients undergoing angioplasty of the left anterior descending coronary artery, we sampled coronary venous blood two and 10 minutes after the last balloon inflation but failed to show any increase in lipid peroxide concentrations. Studies in which coronary venous blood had been sampled immediately after balloon inflation(s) have reported negative and positive results. Recent animal studies have indicated that there is a positive relation between the severity of preceding myocardial ischaemia and the degree of subsequent reperfusion injury due to oxygen radicals. If, in patients undergoing coronary angioplasty, the production of lipid peroxides is related to preceding cycles of ischaemia and reperfusion it may be possible to correlate lipid peroxide concentrations with the degree of ischaemia produced during balloon inflation.

**Patients and methods**

Fifteen patients (age range 42–70; 12 men) with
stable angina pectoris undergoing angioplasty of a stenosis in the proximal left anterior descending coronary artery were studied. Three patients had had previous myocardial infarctions (one anterior, two inferior), all more than 12 months before the study. All regular medications were continued including calcium antagonists (12), β adrenoceptor antagonists (five), nitrates (nine), and 150 mg aspirin daily (15 patients). Blood samples from the great cardiac vein were taken with a 7F Gensini catheter placed through a left antecubital fossa vein. The position of the catheter was confirmed radiologically. All samples were taken after systemic heparinisation. Simultaneous aortic and coronary venous samples of 20 ml were taken before the first inflation and at the end of the procedure. Additional coronary venous samples of 10–12 ml were taken 0, 30, and 60 seconds after each inflation up to a maximum of five inflations per patient. Serial samples were taken to avoid missing the period of peak release of lactate or lipid peroxides. In the final three patients serial aortic and coronary venous blood was sampled simultaneously but only after the first inflation. All inflations were of 60 seconds duration. The protocol was approved by the ethics committee, Glasgow Royal Infirmary and patients gave informed written consent.

ASSAYS
Lipid peroxides were measured in plasma (with added ethylenediaminetetra-acetic acid (EDTA)) by high performance liquid chromatography according to a modification of a previously described method. Many other compounds, including some drugs, can interfere in the thiobarbituric acid assay for lipid peroxides. With high performance liquid chromatography we aimed to reduce such interference compared with older methods that use spectrophotometric or fluorimetric techniques. Mean (SD) concentration from repeated measurements in this assay was 3.8 μM (0–3) giving a coefficient of variation of 8%. Lactate concentration was measured by a commercially available enzymatic assay (Boehringer Diagnostica, Mannheim) with an intra-assay coefficient of variation of 0–7%. The maximum ST segment shift in any of the 12 electrocardiographic leads recorded continuously throughout each balloon inflation was measured manually by an operator who was not aware of the results of the lactate or lipid peroxide measurements.

STATISTICS
The distribution of each variable was assessed by calculating the standardised coefficients of skewness and kurtosis. All of the data were normally distributed and so the summary statistic employed is mean and 95% confidence interval (95% CI). Repeated measures analysis of variance was used to assess the overall statistical significance of the differences between lipid peroxide and lactate concentrations, and the ST segment shift at different times. Thereafter Bonferroni corrected t tests were used. Linear regression analysis was performed to examine the relationship between variables. All statistical analyses were carried out with a PC version of Statgraphics (STSC Inc, Maryland, USA).

Results
The aortocoronary lipid peroxide gradient was 0–2 (0–0 to 0–4) μ mol/l before angioplasty and −0–2 (−0–4 to 0–0) μ mol/l five minutes after the final inflation (p = 0–03). The aortocoronary lactate gradients at the same times were 0–0 (−0–2 to 0–2) μ mol/l and −0–1 (−0–3 to 0–1) μ mol/l (p = 0–86).

Lipid peroxide concentrations in the great cardiac vein
No increases above pre-angioplasty concentrations occurred in two patients. The percentage of samples in the other 13 patients with concentrations above pre-angioplasty values ranged from 25% to 100% per patient. Overall, 82 of 136 (60%) samples assayed were raised. The peak concentration was seen at zero seconds in 32% of inflations, at 30 seconds in 39%, and at 60 seconds in 29%. The peak concentration after each of the first three serial dilatations was significantly greater than both the pre and post-angioplasty samples, which were not different from each other (table 1). The peak percentage increase from pre-angioplasty values exceeded 2 SDs of the relevant measurement error (16%) after 27 out of 46 (59%) inflations (fig 1). There was no significant difference between the peak percentage change after any of the first three serial inflations (table 2).

Figure 1 Individual peak percentage change in the concentration of lipid peroxide in the great cardiac vein (GCV LP) after one to five serial balloon inflations in the left anterior descending coronary artery. The broken line indicates 2 SDs of the error of repeated measurements (see text).
Lactate concentrations in the great cardiac vein
No increases above pre-angioplasty concentrations were seen in any of the samples from one patient. This patient also showed no electrical evidence of ischaemia but did have raised lipid peroxide concentrations. The percentage of samples in the other 14 patients with concentrations above pre-angioplasty values ranged from 33% to 100% per patient. Overall, 104 out of 122 (85%) of samples assayed were raised. The peak concentration was seen at zero seconds in 79%, at 30 seconds in 15%, and at 60 seconds in 6% of cases. The peak concentrations immediately after each of the first three inflations were significantly greater than both the pre and post-angioplasty samples, which were not different from each other (table 1). The peak percentage increase from pre-angioplasty values exceeded 2 SDs of the measurement error (1-4%) after 43 out of 46 (93%) inflations (fig 2). There was no significant difference in the peak percentage changes between any of the first three serial inflations (table 2).

Electrocardiographic changes
There was no significant difference in the maximum ST segment shift occurring between any of the first three serial inflations (table 2).

Correlations
A significant positive correlation was found between peak lactate concentrations in the great cardiac vein and maximum ST segment shift after each balloon inflation. No correlation was shown, however, between peak lactate and lipid peroxide concentrations in the great cardiac vein. There was a significant negative correlation between the peak concentrations of lipid peroxides in the great cardiac vein and maximum ST segment shift (fig 3).

Discussion
This study confirms that raised concentrations of lipid peroxides can be detected in coronary venous blood after angioplasty. It has been suggested that the production and release of lipid peroxides during coronary balloon angioplasty result from free radical mediated damage to polyunsaturated membrane lipids after cycles of myocardial ischaemia and reperfusion.7 Our results suggest that no relation exists between lipid peroxide concentrations in coronary venous plasma after angioplasty and the degree of preceding myocardial ischaemia as assessed by either ST segment shift or lactate production. There are several possible explanations for this finding.

Firstly, we may not have produced a large enough ischaemic insult to initiate reperfusion injury. Aside from the evidence linking the degree of reperfusion injury to the extent of the preceding ischaemia as outlined in the introduction, there is some evidence that only zero flow ischaemia before reflow results in oxygen radical mediated peroxidation of membrane lipids.1513 We cannot determine the

<table>
<thead>
<tr>
<th>No</th>
<th>Lipid peroxides (%)</th>
<th>Lactate (%)</th>
<th>ST segment shift (mm)</th>
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</thead>
<tbody>
<tr>
<td>15</td>
<td>20 (13 to 26)</td>
<td>147 (72 to 222)</td>
<td>0.8 (0.6 to 1)</td>
</tr>
<tr>
<td>11</td>
<td>17 (9 to 25)</td>
<td>141 (54 to 229)</td>
<td>0.9 (0.6 to 1.1)</td>
</tr>
<tr>
<td>10</td>
<td>24 (15 to 32)</td>
<td>167 (70 to 264)</td>
<td>0.9 (0.6 to 1.2)</td>
</tr>
</tbody>
</table>

No significant differences were found between values of any indices after first, second, or third inflations.

| Table 2 Percentage change, mean (95% CI), from pre-angioplasty values of peak lipid peroxide (LP) and lactate concentrations in the great cardiac vein, and maximum ST segment shift after 1–3 serial 60 second balloon inflations in the left anterior descending artery |

<table>
<thead>
<tr>
<th>Dilatation</th>
<th>Change in GCV lactate from baseline (%)</th>
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</thead>
<tbody>
<tr>
<td>1st</td>
<td></td>
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<td>2nd</td>
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<td>3rd</td>
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Figure 2 Individual peak percentage change in lactate concentration in the great cardiac vein (GCV) after one to five serial balloon inflations in the left anterior descending coronary artery.

Figure 3 Simple linear regression analysis of the relationship between: (A) peak lactate concentration in the great cardiac vein (GCV) and maximum ST segment shift; (B) peak GCV lipid peroxide (LP) concentration and maximum ST segment shift; (C) peak GCV LP concentration and lactate concentration.
degree of residual perfusion present during balloon inflation in our patients. Secondy, even if severe or zero flow ischaemia were produced, we cannot be sure that the coronary venous catheter was sampling the effluent from the ischaemic area. The difficulties of assessing regional myocardial metabolism by coronary venous sampling are well known.\textsuperscript{14,15} By sampling far into the great cardiac vein only in patients undergoing angioplasty of relatively proximal left anterior descending artery stenoses we hoped to minimise this potential problem. In 14 out of 15 cases measurements of lactate concentration confirmed that the coronary venous catheter was draining an area of at least partially ischaemic myocardium.

Thirdly, the actual sampling protocol itself may not have enabled the true trans-myocardial release of lipid peroxides to be measured. Serial samples were taken after balloon deflation, but it is possible that even frequent measurements of the concentration of a metabolite may miss the moment of peak production, particularly in the absence of any knowledge of flow. The rapid and dynamic changes in coronary artery blood flow during balloon angioplasty are such that the accurate measurement of the maximum rate of production of any metabolite would require simultaneous and continuous measurement of metabolite concentration and coronary flow.\textsuperscript{16} This is not technically feasible at present. Baseline samples from the great cardiac vein were not taken before each inflation, but for all inflations studied, mean concentrations of both lactate and lipid peroxides were not significantly different from pre-angioplasty concentrations 60 seconds after reperfusion, a finding consistent with earlier data.\textsuperscript{7} Ideally, one should sample simultaneously in the aorta and great cardiac vein to be certain that any changes detected in concentrations in the great cardiac vein are occurring across the coronary circulation. We did this in our final three patients and found, as did other groups, no evidence of any consistent change in the arterial concentrations of either lactate or lipid peroxides during coronary angioplasty.\textsuperscript{2,6}

Finally, the coronary venous effluent may not reflect peroxidation of lipids within the reperfused myocardium. Studies that have measured products of lipid peroxidation in the coronary effluent and in different subcellular fractions of reperfused hearts have indicated that although there is a small rise in the lipid peroxide content of the coronary effluent after reperfusion, myocardial lipid peroxidation occurs predominantly in the membranes of certain intracellular organelles.\textsuperscript{13,17} It is doubtful whether these lipid peroxides are free to cross the cell membrane and extracellular space and enter the circulation.

Notwithstanding these considerations the lack of any positive correlation between either the electrical or biochemical evidence of ischaemia and the raised lipid peroxide concentrations suggests that production of these can be, at best, only partly due to damage mediated by oxygen radicals after post-ischaemic reflow. Other possible sources include the mechanically disrupted atherosclerotic plaque, which is known to contain substantial amounts of lipid peroxides,\textsuperscript{18,19} platelets,\textsuperscript{20,21} leucocytes, and endothelial cells. These cells are all capable of producing prostaglandins, or leukotrienes, or both, and peroxides may be formed during their biosynthesis.\textsuperscript{22,23} Even though all of our patients were taking aspirin, which interferes with the synthesis of peroxides by the cyclooxygenase enzyme system,\textsuperscript{24} it is unlikely that this pathway was completely inactivated.

Irrespective of their origin, what consequences might the release of these lipid peroxides have? Lipid peroxides can selectively inhibit prostacyclin (PGI\textsubscript{2}) synthesis\textsuperscript{24} and through effects on antithrombin III activity\textsuperscript{25,26} induce thrombin generation in normal plasma.\textsuperscript{27} These prothrombotic effects could play a part in the pathogenesis of both acute occlusion and late restenosis after coronary angioplasty. Studies are needed to explore these potential mechanisms of disease and to identify the origin of the increased lipid peroxide concentrations found after angioplasty.

A secondary conclusion from the results of this study is that when 60 seconds balloon inflations are used, the degree of myocardial ischaemia produced during angioplasty is similar after at least three serial inflations. In animal models short earlier periods of coronary occlusion can protect against ventricular arrhythmias during a subsequent sustained ischaemic insult.\textsuperscript{28,29} This is known as ischaemic preconditioning. The exact timing of the sequences of ischaemia and reperfusion necessary to produce preconditioning and the underlying mechanisms are not clear. Several groups have used the situation of coronary angioplasty to explore this phenomenon in humans. Our finding of an absence of preconditioning with 60 second coronary occlusions is consistent with the results of three previous studies.\textsuperscript{30,31,32} Other workers using 90 second inflations have shown a lesser degree of ischaemia after a second inflation.\textsuperscript{33,34}

Differences in patient selection, the method of quantifying ischaemia, and the degree of collateral flow during balloon occlusion may go a long way towards explaining these opposing results. Alternatively, it may be the case that there is a critical occlusion time needed to initiate the preconditioning mechanism, this time being greater than 60 seconds. The results of further studies specifically designed to consider these questions are awaited with interest.

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4. Paterson JB, Oldroyd KG, Rumley AG, et al. Free radical...
Coronary venous lipid peroxide concentrations after coronary angioplasty: correlation with biochemical and electrocardiographic evidence of myocardial ischaemia. K G Oldroyd, J R Paterson, A G Rumley, H Eteiba, A P Rae, J Shepherd, S M Cobbe, I Hutton (July issue, volume 68: pages 43–7.) There were several editorial mistakes in figure 3 of this paper, for which we apologise. A correct version is shown on the right.

**Figure 3** Simple linear regression analysis of the relation between (A) peak lactate concentration in the great cardiac vein (GCV) and maximum ST segment shift; (B) peak GCV lipid peroxide (LP) concentration and maximum ST segment shift; (C) peak GCV lactate concentration and LP concentration.