Thrombolytic treatment in acute myocardial infarction: neutrophil activation, peripheral leucocyte responses, and myocardial injury

Kulasegaram Ranjadayalan, Velaitham Umachandran, Simon W Davies, Denise Syndercombe-Court, Charles N Gutteridge, Adam D Timmis

Abstract
Objective—To examine early leucocyte responses and neutrophil activation in acute myocardial infarction treated by streptokinase and to relate the findings to coronary recanalisation and indices of myocardial damage in order to provide further information about the role of neutrophils in the evolution of injury.

Design—Group analysis of paired blood samples, obtained before streptokinase treatment and one hour after it, and of three indirect measures of myocardial injury: left ventricular ejection fraction, QRS score, and peak creatine kinase.

Setting—The coronary care unit of a district general hospital.

Patients—39 patients with acute myocardial infarction who underwent paired blood sampling (before streptokinase and one hour after streptokinase) and cardiac catheterisation 5 (3–8) days later.

End points—Changes in peripheral white cell and neutrophil counts and plasma elastase one hour after streptokinase infusion. Comparison of these variables in patients with and without patency of the infarct related coronary artery. Correlations between these variables and indirect measures of myocardial injury.

Results—Neutrophil activation, as reflected by plasma elastase, increased sharply one hour after streptokinase. Total white cell and neutrophil counts also increased. Changes tended to be more pronounced in patients with patency of the infarct related artery, though the trend was not statistically significant. Neutrophil activation before streptokinase was unrelated to indirect indices of myocardial injury but only one hour after streptokinase a weak negative correlation with left ventricular ejection fraction had developed. Peripheral neutrophil responses showed a similar relation to ejection fraction and also correlated with peak creatine kinase and QRS score.

Conclusions—Thrombolytic treatment in acute myocardial infarction is associated with an abrupt reactive neutrophil response which provides an early measure of injury. It is also associated with neutrophil activation, probably in response to coronary recanalisation and myocardial reperfusion. Activated neutrophils are recognised as mediators of reperfusion injury in experimental infarction and the data in the present study provide preliminary evidence of a similar pathogenic role in the clinical setting.

The benefits of thrombolytic treatment for reducing mortality in acute myocardial infarction are well established but residual left ventricular dysfunction usually persists even when thrombolysis is successful in restoring coronary patency. Residual left ventricular dysfunction largely reflects damage incurred during the ischaemic period but damage at the time of reperfusion can also contribute.

We have previously reported evidence that oxygen derived free radicals are generated in response to myocardial reperfusion in patients treated with streptokinase and more recently we have provided preliminary data relating the free radical response to eventual infarct size. Activated neutrophils are a potent source of oxygen derived free radicals and there is a large body of experimental data indicating that they may be important in the pathogenesis of reperfusion injury. Neutrophil infiltration of the infarct territory can be shown within three to six hours of coronary occlusion and is particularly pronounced after reperfusion. Reparfusion provokes various chemotactic mechanisms including the complement system and coagulation pathways that activate neutrophils and lead to release of oxygen derived free radicals, arachidonic acid metabolites, and lysosomal enzymes all of which are toxic to vascular endothelium and cardiac myocytes and may contribute to the extension of myocardial injury. Evidence for the pathogenic role of neutrophils in experimental reperfusion injury is strengthened by the demonstration of myocardial protection with neutrophil deplete perfusates or agents that suppress neutrophil chemotaxis.

Despite the body of experimental data implicating neutrophils as mediators of reperfusion injury, clinical data are scarce and the importance of this mechanism in patients with acute myocardial infarction is unknown. The purpose of the present study, therefore, was to
Neutrophil activation in acute myocardial infarction

Table 1  Data on patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>39</td>
</tr>
<tr>
<td>Age (years) (median (range))</td>
<td>57 (47-67)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>29</td>
</tr>
<tr>
<td>Female</td>
<td>10</td>
</tr>
<tr>
<td>Infarct location:</td>
<td></td>
</tr>
<tr>
<td>Anterior</td>
<td>18</td>
</tr>
<tr>
<td>Inferior</td>
<td>21</td>
</tr>
<tr>
<td>History of previous infarction</td>
<td></td>
</tr>
<tr>
<td>Time to streptokinase (min) (median (range))</td>
<td>5 (210 (165-348))</td>
</tr>
<tr>
<td>Time to cardiac catheterisation (days) (median (range))</td>
<td>5 (3-8)</td>
</tr>
<tr>
<td>Infarct related coronary artery:</td>
<td></td>
</tr>
<tr>
<td>Recanalised</td>
<td>34</td>
</tr>
<tr>
<td>Non-recanalised</td>
<td>5</td>
</tr>
<tr>
<td>Coronary artery disease:</td>
<td></td>
</tr>
<tr>
<td>1 Vessel</td>
<td>16</td>
</tr>
<tr>
<td>2 Vessel</td>
<td>10</td>
</tr>
<tr>
<td>3 Vessel</td>
<td>13</td>
</tr>
<tr>
<td>Left ventricular ejection fraction (%) (median (range))</td>
<td>57 (47-67)</td>
</tr>
</tbody>
</table>

Examine the interaction of thrombolytic treatment and neutrophil activation in patients with acute myocardial infarction and to relate the findings to coronary recanalisation and indices of myocardial damage in order to provide further information about the role of neutrophils in the pathogenesis of reperfusion injury.

Patients and methods

Patients

We studied a consecutive series of 39 patients with acute myocardial infarction receiving thrombolytic treatment in whom we obtained a complete set of paired blood samples for white cell and neutrophil counts and plasma neutrophil elastase (table 1). All were treated with 1·5 MU of streptokinase infused intravenously over one hour. The median time from onset of continuous severe chest pain to start of streptokinase infusion was 210 (165-348) minutes.

Blood sampling and analysis

Paired venous blood samples were obtained immediately before thrombolytic treatment and again one hour after the streptokinase infusion. Samples were drawn without venous occlusion and anticoagulated in EDTA for measurement of total leucocyte and neutrophil counts and in citrate for measurement of plasma neutrophil elastase. Leucocytes and neutrophils were counted by a Coulter S + V1 automated blood cell counter. Samples for plasma neutrophil elastase were centrifuged and the plasma frozen to −20°C within 30 minutes of the blood being drawn. The plasma samples were stored at −80°C for analysis in one batch. Neutrophil elastase was measured as elastase-α proteinase inhibitor complexes by a sensitive double antibody sandwich ELISA (Merck kit 15689 Immunoassay Polymorphonuclear Elastase). The detection limit of the test is approximately 0·25 ng elastase in complex bound form.

Electrocardiographic and enzymatic data

Predischarge 12 lead electrocardiograms adequate for QRS scoring were obtained in 27 patients seven (range 5-9) days after admission. Scoring was by the 29 point system described by Palmeri et al21 on the basis of the Q and R wave duration and the R to Q and R to S amplitude ratios. Blood samples for creatine kinase activity were obtained on admission to hospital, and on the next two consecutive mornings according to the policy of our coronary care unit. A complete set of three creatine kinase measurements was obtained in 29 patients.

Cardiac catheterisation

All patients gave written informed consent for coronary arteriography and single plane (right anterior oblique) left ventriculography. Studies were performed five (range 3-8) days after hospital admission to determine the patency of the infarct related coronary artery, the extent of associated coronary artery disease, and left ventricular function. Patency of the infarct related coronary artery was assessed by TIMI criteria.2 Left ventriculograms suitable for quantitative analysis of ejection fraction by the method of Sandler and Dodge22 were obtained in 31 patients. Of these, four had a history of previous Q wave infarction, leaving 27 studies suitable for assessment of myocardial injury in the present study.

Statistical analysis

In all analyses, non-parametric (distribution free) statistics were used. Values were expressed as median with the interquartile range in parentheses. For unpaired comparisons we used the Mann-Whitney U test and for paired comparisons the Wilcoxon test. We used the Spearman rank correlation coefficient to examine correlations.

Results

Baseline values (table 2)

Blood samples obtained at the time of admission, before treatment with streptokinase, showed modest increases in mean white cell and neutrophil counts. Plasma neutrophil elastase was also increased but showed no significant relation with the neutrophil count.

Responses to streptokinase (table 2)

Coronary arteriography seven (5) days (mean (SD)) after streptokinase infusion showed that the infarct related artery was patent in 87% of patients. Only five patients had persistent coronary occlusion. The total white cell and neutrophil counts increased sharply an hour after streptokinase, with median values increasing by 43% and 69% respectively. A 49% increase in plasma elastase indicated
neutrophil activation. These changes were all highly significant but no differences could be shown between patients with a patent infarct related vessel and those without at five (3–8) days. Nevertheless, in those with early patency, white cell and peripheral neutrophil responses tended to be more vigorous, as did neutrophil activation (median values for plasma elastase increased by 32% after streptokinase infusion in patients with early coronary patency but showed no change in patients with persistent coronary occlusion (table 3).

NEUTROPHILS, NEUTROPHIL ELASTASE, AND MYOCARDIAL INJURY

The neutrophil count on admission, before streptokinase infusion, was unrelated to any of the indirect measures of myocardial injury. Only one hour after streptokinase, however, significant correlations with left ventricular ejection fraction, peak creatine kinase, and QRS score had developed (fig 1). Neutrophil activation, as reflected by plasma elastase, also correlated with left ventricular ejection fraction one hour after streptokinase infusion but not with the other indirect measures of myocardial injury (fig 2).

Discussion

Studies in which autologous neutrophils labelled with indium-111 were reinjected into patients with acute myocardial infarction indicated that the stimulus for neutrophil activation occurs early.23 24 This is confirmed in the present study which showed an abrupt increase in plasma elastase within an hour of streptokinase infusion. The close temporal relation with administration of thrombolytic treatment suggests that reperfusion may be important in the activation process. Nevertheless, the trend towards a more vigorous elastase response in patients with patency of the infarct related artery was not statistically significant, possibly because of numerical imbalance between the groups (there were only five “occluded” cases) or delay before coronary arteriography. This will have led to random misclassification of some “patent” and “occluded” cases, so reducing the power of the statistical analysis.

Others reported increases in plasma elastase after thrombolytic treatment of acute infarction,25 26 Bell et al, however, found no associated increase in the uptake of111 In-labelled neutrophils in the infarcted myocardium and concluded that the elastase response was due to clot lysis or intracoronary (as opposed to intra-myocardial) activation of neutrophils.25 In experimental models of coronary occlusion, however, activated neutrophils accumulated very rapidly in the zone of infarction during reperfusion,27 and if this occurs clinically in response to successful thrombolytic therapy it may well have contributed to the sharp increase in plasma elastase seen in this and other studies. Activated neutrophils were identified as a cause of reperfusion injury in experimental infarction and the present study suggests they may have a similar effect after thrombolytic treatment. Thus we were able to show a significant negative correlation between plasma elastase one hour after streptokinase and left ventricular ejection fraction; in patients with a more exaggerated elastase response left ventricular function was significantly more impaired. This indicates that neutrophil activation was, at least in part, related to the process of infarction and was not stimulated directly by streptokinase. Nevertheless, the correlation was weak and there was no significant relation with peak creatine kinase or QRS score, indicating that the contribution of neutrophil activation to infarction is likely to be small, particularly when compared with the effects of ischaemic injury.

An alternative explanation for the sharp rise in plasma elastase early after streptokinase infusion is washout from a previously unperfused myocardium. This would imply considerable accumulation of activated neutrophils in the zone of infarction early after coronary occlusion rather than their entry in response to reperfusion. A study such as ours

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>Recanalised artery (n = 34)</th>
<th>Not recanalised artery (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White cells (x10^9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before streptokinase</td>
<td>11.8 (9.7–13.9)</td>
<td>11.0 (9.3–11.3)</td>
</tr>
<tr>
<td>After streptokinase</td>
<td>17.4 (12.6–20.8)</td>
<td>15.1 (9.6–16.4)</td>
</tr>
<tr>
<td>Difference</td>
<td>5.2 (2.1–7.8)</td>
<td>5.9 (0.7–4.9)</td>
</tr>
<tr>
<td>Neutrophils (x10^9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before streptokinase</td>
<td>8.4 (6.1–11.1)</td>
<td>8.4 (6.9–8.5)</td>
</tr>
<tr>
<td>After streptokinase</td>
<td>14.9 (10.1–18.6)</td>
<td>11.3 (8.0–13.9)</td>
</tr>
<tr>
<td>Difference</td>
<td>6.2 (3.2–9.3)</td>
<td>2.8 (1.6–5.8)</td>
</tr>
<tr>
<td>Plasma neutrophil elastase (ng/ml):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before streptokinase</td>
<td>59.9 (43.3–94.3)</td>
<td>53.5 (42.7–74.0)</td>
</tr>
<tr>
<td>After streptokinase</td>
<td>81.6 (56.1–176.0)</td>
<td>66.3 (45.2–81.6)</td>
</tr>
<tr>
<td>Difference</td>
<td>19.2 (0.84–2.1)</td>
<td>0 (–12.8–28.7)</td>
</tr>
</tbody>
</table>

None of the differences between the group with recanalised arteries and the group without was statistically significant.
cannot distinguish between these separate mechanisms, but experimental data indicate that substantial intramyocardial accumulation of activated neutrophils early after coronary occlusion is not seen in the absence of reperfusion, when an explosive influx occurs.21,22 Moreover, during angioplasty very brief periods of coronary occlusion followed by reperfusion produce a sharp increase in plasma elastase which cannot be explained by washout.23 Thus washout is unlikely to account for the elastase response seen in the present study and certainly could not account for the increase in circulating neutrophils which was too rapid and too large to have derived from the zone of infarction.

Peripheral leucocytosis in acute myocardial infarction peaks after three to four days and is usually regarded as a component of the generalised stress response.24 However, this is unlikely to explain the abrupt rise in circulating neutrophils that occurred immediately after thrombolytic treatment in the present study. Streptokinase infusion might itself have contributed to the neutrophil response but cannot have been the major determinant because responses were significantly related to three independent measures of myocardial injury, suggesting that streptokinase induced reperfusion of the ischaemic territory caused a detectable leucocytosis in proportion to the degree of injury.

Quantification of myocardial injury and infarct size is difficult and although ejection fraction, peak creatine kinase, and QRS score are widely used,25 32 clinical constraints imposed limitations on our methods. Thus a series of only three blood samples per patient was available for determining the peak creatine kinase activity, and the ejection fraction was measured before hospital discharge in most patients; reversible myocardial stunning may have made a variable contribution to contractile dysfunction.33 Nevertheless, despite these limitations, relations between neutrophil responses early after thrombolytic treatment and these three independent indices of infarct size showed remarkable consistency, supporting our hypothesis that the neutrophil response provides an early measure of myocardial injury.

We found that thrombolytic treatment in acute myocardial infarction leads to an abrupt increase in circulating neutrophils which provides an early measure of injury. It also leads to neutrophil activation, probably in response to coronary recanalisation and myocardial reperfusion. Activated neutrophils are recognised as mediators of reperfusion injury in experimental infarction and the data in the present study provide preliminary evidence of a similar pathogenic role in the clinical setting.

PLANTS IN CARDIOLOGY

Ergometrine (ergonovine)
The fungus *Claviceps purpurea* (Clavicipitaceae) is a parasite that infects the flowers of cereals, notably rye, replacing them with a curved hard mass or sclerotium of mycelia which is called ergot (from Old French argot, a cock’s spur). In the Middle Ages rye bread infected with ergot caused large epidemics of gangrene of the hands and feet and mental symptoms. Ergotism was often fatal. The blackened extremities looked as if they had been burnt in a fire, and the malady was called St Anthony’s Fire because the patients were treated at the saint’s monastery in Padua.

Since 300 BC ergot has ergot has been recognised as causing abortion. Thus its vasoconstrictor, oxytocic, and cerebral effects were known long before the responsible alkaloids were isolated. Ergotoxine and ergotamine were the first to be found and their effect on human uterine contraction was studied in 1932 by Chassar Moir. He then tested the traditional liquid extract of ergot which was thought by some scientists, though not by clinicians, to be ineffective and he showed its action to be much larger than the two alkaloids. This led to the isolation of a new alkaloid, ergometrine (ergonovine). Its vasoconstrictor action was first used in the investigation of coronary artery spasm in 1976 by T O Cheng at the suggestion of E Shirey and W Sheldon.

Ergot has been called “a veritable treasure house of pharmacological constituents.” Ergot derivatives include the hallucinogen, lysergic acid diethylamide (LSD), bromocriptine (used to treat pituitary tumours and parkinsonism), and the serotonin antagonist, methysergide, which is an effective prophylactic in migraine. Methysergide can cause fibrosis of the mitral and aortic valves.

There are over 200 000 species of fungi. They are of great importance in medicine, producing antibiotics and also cyclosporin. The fungi are by tradition classified as plants but because they differ from them and from animals in several ways there is a proposal to create a separate fungal kingdom.

A HOLLMAN
Thrombolytic treatment in acute myocardial infarction: neutrophil activation, peripheral leucocyte responses, and myocardial injury.

K Ranjadayalan, V Umachandran, S W Davies, D Syndercombe-Court, C N Gutteridge and A D Timmis

*Br Heart J* 1991 66: 10-14
doi: 10.1136/hrt.66.1.10

Updated information and services can be found at:
http://heart.bmj.com/content/66/1/10

These include:

**Email alerting service**

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Notes**