Patterns of myoglobin release after reperfusion of injured myocardium

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ABSTRACT Myoglobin is an intracardiac protein that is released into the blood after myocardial injury and is then cleared rapidly by the kidneys. This study was undertaken to determine whether successful reperfusion of damaged myocardium could be assessed by examination of blood myoglobin concentration-time patterns. After release of a 2 hr occlusion of the mid left anterior descending coronary artery in 11 dogs that had been instrumented over the long term, immunoreactive arterial plasma concentration of myoglobin, [Mb], rose rapidly to a peak within 25 ± 2(SEM) min (range 20 to 40). Individual peaks were three to 165 times the myoglobin levels immediately before release of the occlusion. Myoglobin was cleared rapidly from plasma, falling to one-half its peak level 38 ± 3 min after the peak. Similarly well-defined peaks in [Mb] were evident in plasma from the great cardiac vein (GCV), with a mean time to peak of 16 ± 2 min and a magnitude of two to 177 times prerelease values. In contrast, arterial and GCV creatine kinase activity-time curves showed less defined peaks and they occurred later and with more variability (60 to 330 min after reperfusion). In nine patients with acute infarction, successful coronary artery reopening was also accompanied by a sharp four- to sixteenfold rise in plasma [Mb] within 1 to 2 hr. Elevations in plasma creatine kinase were slower and more prolonged, peaking at 2 to 18 hr. The sharp, early peaks in [Mb] after successful reperfusion were in contrast to the case in one patient in whom reperfusion was not successful and in whom there was only a gradual increase in [Mb], T_{25-100}, defined as the time required for [Mb] to rise from 25% to 100% of peak, averaged 48 ± 9 min for the nine patients undergoing successful reperfusion. In contrast, T_{25-100} in published series of patients with acute infarction in whom reperfusion was not attempted averaged 5 to 6 hr. In summary, reperfusion of injured myocardium results in a rapid release of myoglobin into the blood in both experimental animals and man. An early peak in the myoglobin concentration-time curve appears to be an additional useful indicator of reopening of the vessel when reperfusion is undertaken and a coronary arteriogram is not available.


IT IS NOW generally agreed that most transmural myocardial infarctions are associated with fresh thrombi superimposed on atherosclerotic lesions of varying severity.¹ Although the thrombi can often be dissolved with lytic agents, it is frequently difficult to assess whether or not reperfusion has occurred if an immediate coronary arteriographic examination is not performed. Some authors have attempted to use the rate of rise, peak magnitude, and/or time to peak creatine kinase (CK) elevation after reperfusion, but there remains substantial overlap in these parameter values in patients undergoing successful and those undergoing unsuccessful reperfusion.²⁻⁶ Myoglobin is a cardiac protein the levels of which are frequently elevated in plasma of patients with acute infarction and these elevated levels are often apparent before CK is detected.⁷ Myoglobin is known to be cleared from the plasma with a half-life of disappearance of less than 10 min.⁸ Because of this early appearance and unusually rapid clearance, blood myoglobin concentration-time patterns may provide a useful marker of entry of protein into the vascular space in situations such as myocardial reperfusion.

The present study was undertaken to determine whether successful reperfusion of damaged myocardium could be appreciated by examination of blood myoglobin concentration-time patterns. The problem
was initially addressed by measuring myoglobin entry into and disappearance from plasma after a period of acute myocardial injury in dogs. The protocol was intended to simulate the procedure for lysis of intracoronary thrombi now being performed clinically. Subsequently, patients undergoing reperfusion with intra-coronary streptokinase, percutaneous transluminal coronary angiography (PTCA), and intravenous streptokinase were also studied.

Methods

Fourteen mongrel dogs weighing 15 to 26 kg were anesthetized with sodium pentobarbital and ventilated with a Harvard positive-pressure respirator. Under sterile conditions, a left thoracotomy was performed in each, and one or two pneumatic occluders were placed on the left anterior descending coronary artery (LAD) just distal to the first or second diagonal branch. Electrodes were sewn onto the epicardium in the distributions of the LAD and the undisturbed left circumflex coronary artery (LC). A catheter was placed in the descending aorta for measurement of arterial pressure and subsequent withdrawal of blood samples for determination of systemic arterial myoglobin and CK levels. All animals were allowed to recover for at least 7 days so that elevated myoglobin and CK levels secondary to skeletal muscle damage could return to baseline.

On the day of the short-term study, each animal was anesthetized with thiopental sodium and halothane, and a cutdown was performed on the external jugular vein. A catheter was placed in the coronary sinus under fluoroscopic control and advanced into the great cardiac vein (GCV), thereby allowing selective sampling from the area of injury in the LAD. Aortic pressure was measured throughout the experimental period, as were lead II of the standard electrocardiogram and the LAD and the LC surface electrograms. After the administration of 1000 units iv heparin, three sets of control arterial and GCV samples for determination of myoglobin concentration, [Mb], and CK activity were obtained. Intravenous lidocaine (25 mg) was administered, and the LAD occluder(s) was inflated for a 2 hr period. One animal developed ventricular fibrillation during the period of occlusion and was not studied further. In two animals the occluders were not inflated. In nine of the remaining 11 animals, the LAD surface electrogram showed ST segment shifts, loss of R wave voltage, and/or widening of the QRS during LAD occlusion.

Arterial and coronary venous samples for determination of myoglobin and CK levels were obtained at 30 min intervals during the 2 hr period of occlusion. At the end of the period, an additional 25 mg of lidocaine was administered intravenously and the occluder(s) was released. Simultaneous arterial and coronary venous samples were then drawn at 1 min intervals for 3 min, at 2 min intervals for 12 min, at 5 min intervals for 25 min, at 10 min intervals for 20 min, and then at 20 min intervals until the completion of the study. In seven animals, sampling was continued for 4 hr after release of the occluder(s); in four other animals, it was continued for 11 hr.

Standard radioimmunoassay and enzymatic methods were used to determine [Mb] and CK activity in each sample. Myoglobin concentration-time curves and CK activity-time curves were analyzed in terms of peak values and time to peak levels. In addition, the half-life of disappearance of arterial myoglobin was determined from the initial portion of the washout phase of the arterial myoglobin concentration-time curve. All values are reported as mean ± SEM unless otherwise stated.

Clinical studies. Ten patients with typical clinical and electrocardiographic evidence of acute myocardial infarction of less than 6 hr duration were studied after giving informed consent. In seven patients, angiography was performed immediately. The artery supplying the area of infarction was totally occluded in four patients, and was estimated to be 99% narrowed in the remainder. Reperfusion was achieved in six patients after administration of intracoronary streptokinase and/or PTCA and was documented by angiography. Neither measure resulted in reperfusion in the seventh patient. Three additional patients were each treated with 1,000,000 units of intravenous streptokinase, which was infused over ~30 min. Each experienced resolution of chest discomfort and ST elevation within 90 min of completion of the infusion. The vessel supplying the area of acute infarction was subsequently demonstrated to be patent at angiography performed 1, 4, and 22 days later.

Venous blood samples for determination of myoglobin levels and CK activity were obtained over an interval of 5 to 60 min before and for at least 3 hr after the reperfusion procedure. Plasma [Mb] was determined with a radioimmunoassay kit developed for human plasma (NMS Pharmaceuticals, Inc., Newport Beach, CA). Plasma CK activity was determined with a commercial kit (SIGMA 45-UV) based on the hexokinase/glucose-6-phosphate dehydrogenase enzyme coupled system.

Results

Animal studies. Myoglobin concentration-time curves were analyzed for the 11 animals completing the occlusion protocol. Although only nine animals showed surface electrographic changes during occlusion, all 11 had clear-cut elevations in myoglobin and CK levels. Hemodynamic parameters remained constant throughout the experimental period in all 11 animals (table 1).

Figure 1 illustrates typical arterial myoglobin and CK concentration-time curves from one animal. [Mb] rose rapidly within a minute of release of occlusion, peaking at 7262 ng/ml 30 min later. The rapid clearance of myoglobin is evident from the disappearance phase of the concentration-time curve, with the concentration falling to one-half of its peak value ~30 min after the peak. The CK activity-time curve also showed a marked increase in enzyme activity after release of occlusion, but the rate of rise was slower and the peak value of 4181 IU/liter occurred ~2 hr after release.

In the two animals in which no electrocardiographic changes were present during LAD occlusion, the asso-

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Summary of hemodynamic data</th>
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<tr>
<td>Early occlusion</td>
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<tr>
<td>After release of occlusion</td>
<td>114 ± 10.6</td>
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<td>End of study</td>
<td>116 ± 9.0</td>
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All values are expressed as mean ± SEM.
FIGURE 1. Myoglobin concentration-time curve (top) and CK activity-time curve (bottom) from one animal (dog 9, tables 2 and 3) before, during, and after release of a 2 hr occlusion of the mid LAD occlusion. See text for details.

ciated myoglobin and CK peak levels were smaller than in the other animals (dogs 6 and 8, tables 2 and 3; figure 2). The small peaks and absence of electrocardiographic changes suggest that only small zones of injury were produced by LAD occlusion (possibly because of preexisting collateral vessels in the canine species).

Figure 3 summarizes arterial myoglobin and CK kinetics for all 11 animals. Peak [Mb] occurred between 20 and 40 min after release of occlusion, with an average time to peak of 25 ± 2 min. This is in contrast to the consistently later peaks for CK, which occurred between 1 and 5½ hr after release of occlusion. An additional finding of interest was that [Mb] fell by 50% of its peak value at an average of 63 ± 4 min after reperfusion, that is, 38 min after its peak.

Figure 4 illustrates transcoronary venous-arterial differences for myoglobin and CK in one animal. At the end of the 2 hr occlusion of the LAD, GCV [Mb] was slightly but distinctly elevated, indicating release of the protein into coronary venous blood during the ischemic period. After release of occlusion, there was a rapid rise in both arterial and GCV [Mb], the former peaking at 3724 ng/ml, and the latter peaking fivefold higher, at 17,502 ng/ml. An additional feature of the myoglobin concentration-time curves illustrated in figure 4 was the persistent coronary venous-arterial concentration difference of ~100 ng/ml. Similar persistent coronary venous-arterial concentration differences were present in all 11 animals and averaged 153 ± 33 ng/ml 3 hr after release of occlusion.

Figure 4 also illustrates simultaneous GCV and arterial CK activity as a function of time. During the ischemic period, there was a slight rise in arterial and coronary venous CK activities; a rapid rise in both followed release of occlusion. In contrast to the myoglobin concentration-time curves, there was no clear persistent GCV-arterial concentration difference. Three hours after release of occlusion, mean GCV-arterial CK differences for all 11 animals averaged 32 ± 27 IU/liter (which is not statistically different from zero).

For the two animals in which the occluders were not

TABLE 2
Arterial and GCV myoglobin data in 11 animals subjected to a 2 hr occlusion of the LAD

<table>
<thead>
<tr>
<th>Dog No</th>
<th>Baseline (ng/ml)</th>
<th>End-occlusion (ng/ml)</th>
<th>Peak (ng/ml)</th>
<th>Time to peak (min)</th>
<th>t½ (min)</th>
<th>Baseline (ng/ml)</th>
<th>End-occlusion (ng/ml)</th>
<th>Peak (ng/ml)</th>
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\(t½ = \text{half-life.}\)
inflated, both myoglobin and CK values remained at control levels (with [Mb] < 42 ng/ml and CK level < 43 IU/liter) throughout the experimental period.

Clinical studies. Figure 5 contrasts myoglobin and CK curves from one patient who underwent successful reperfusion with PTCA with those from the one patient in whom immediate reperfusion was unsuccessful. Rapid increases in myoglobin levels after reperfusion were apparent in each of the six patients in whom intracoronary streptokinase and/or PTCA was successful (figure 6). Peak myoglobin values (1130 to 7200 ng/ml) occurred 49 ± 8 min after the onset of reperfusion and represented a four- to 16-fold increment above the levels observed immediately before reperfusion. CK levels, which rose five- to 42-fold, peaked noticeably later at 11 ± 2 hr after reperfusion.

Data from the patients who received intravenous streptokinase are summarized in figure 7. In each case

<table>
<thead>
<tr>
<th>Dog No</th>
<th>Arterial Baseline (IU/l)</th>
<th>End-occlusion (IU/l)</th>
<th>Peak (IU/l)</th>
<th>Time to peak (min)</th>
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FIGURE 2. Simultaneous arterial and GCV myoglobin (left) and CK (right) concentration-time curves from an animal that showed no electrocardiographic changes during the LAD occlusion (dog 6, tables 2 and 3). Although peak [Mb] is low, an abnormal pattern is clearly evident. A persistent GCV-arterial [Mb] gradient is present, confirming the release of myoglobin from the myocardium. Although a rise in CK activity is also noted, a clear peak is not evident during the period of observation.
there was an abrupt six- to 14-fold rise in [Mb], with peaks occurring 95 to 105 min after completion of the streptokinase infusion. CK activities also rose 23- to 53-fold, with peak levels occurring between 4 and 14 hr after completion of the infusion.

Thus, clinical reperfusion was consistently accompanied by a rapid rise in systemic venous [Mb], with the peak concentration occurring less than 2 hr after therapy. Substantial elevations in CK also occurred with reperfusion, but peak values occurred several hours later.

**FIGURE 3.** Time-to-peak [Mb] (left) and CK activity (right) after release of occlusion in all 11 animals. In contrast to the CK peaks, peak [Mb] occurred 25 ± 2 min after release of occlusion and then fell to one-half peak value 38 ± 3 min later (middle).

**Discussion**

This study demonstrates that myoglobin, a protein that is present in both skeletal and cardiac muscle, is rapidly released into the coronary venous effluent after successful reperfusion of injured myocardium. In all 11 animals successfully undergoing coronary artery occlusion and reperfusion, blood [Mb] increased rapidly after reopening of the vessel, with systemic arterial peaks occurring at 20 to 40 min. These early arterial peaks were followed by a rapid washout of the protein, with half-times of disappearance of 38 ± 3 min. The early peaks in [Mb] contrast with myoglobin concentration-time patterns in animals that sustained myocardial injury but did not undergo reperfusion. In these dogs peak [Mb] occurred at 4 to 7 hr after onset of occlusion. 10, 11 The early peaks and rapid washout of myoglobin also contrast with the patterns of CK activity. Although CK also entered the blood stream rapidly after successful reperfusion of injured myocardium, systemic peaks of this substance occurred significantly later and with greater variability than those of myoglobin, at 1 to 5½ hr after reperfusion.

Similar findings were present in the nine patients who underwent successful coronary reperfusion for acute myocardial infarction produced by arterial occlusions of 99% to 100%. In each case, plasma [Mb] rose rapidly after successful vessel reopening, and each peak level was followed by rapid plasma washout. These findings contrast with the simultaneous CK activity-time curves, which showed a discernible rise.

**FIGURE 4.** Simultaneous arterial and GCV myoglobin concentration-time curves (left) and CK activity-time curves (right) from one animal (dog 12, tables 2 and 3). See text for details.
FIGURE 5. Systemic plasma myoglobin concentration-time curves and CK activity-time curves for two patients undergoing thrombolytic therapy for acute myocardial infarction. In the patient in whom reperfusion was successful, plasma [Mb] rose rapidly after PTCA, peaking at 32 min. CK activity also showed a prominent rise but did not reach its peak of 2360 IU/liter until 18 hr. In the patient in whom reperfusion was not successful, plasma [Mb] rose slowly over 7 hr, achieving a maximum level of 1030 ng/ml.

after reperfusion, but peaked at 2 to 18 hr rather than at 1 to 2 hr. Thus, myoglobin is rapidly released into the systemic circulation and is then rapidly cleared, making its early peak more apparent than that of CK after successful coronary arterial reperfusion. With the use of currently available rapid assays, myoglobin levels can be determined within a few hours after a sample is obtained.

FIGURE 6. Summary of myoglobin concentration-time data for six patients undergoing successful reperfusion with intracoronary streptokinase and/or PTCA. Time of reperfusion is designated time 0. Plasma [Mb] is plotted on the vertical axis as percent of peak myoglobin concentration. See text for details.

FIGURE 7. Summary of myoglobin concentration-time data for three patients in whom reperfusion with intravenous streptokinase was thought to be successful. Data are plotted as in figure 6. Completion of streptokinase is noted as time 0. See text for details.

The relatively rapid disappearance of myoglobin from plasma has been documented in earlier studies. After intravenous administration of 125I-radiolabeled myoglobin to normal dogs, arterial immunoreactive [Mb] decreased monoexponentially over a two-decade range, with a half-time of disappearance of 8.9 ± 1.5(SD) min. This half-time after bolus administration contrasts with the half-time of disappearance of 38 ± 3 min found in the dogs included in the present study. Although a decrease in renal perfusion could theoretically have contributed to the difference, there is no reason to expect an important decrease in renal flow in the face of continuing normal hemodynamics (table 1). A more likely explanation is that there is continuing protein release into the vascular space from damaged myocardium during the first few hours after reperfusion. As discussed above and illustrated in figure 4, coronary venous-arterial myoglobin concentration differences persisted throughout the experimental period in all 11 animals that underwent reperfusion. It is also of interest that myoglobin levels peaked 20 to 40 min after vessel reopening (figure 3) rather than at 1 to 2 min as noted when myoglobin was injected as a bolus. Because coronary flow was not measured in the present study, we are unable to quantify the magnitude of this continuing protein release.

Mechanisms for continuing myoglobin release remain speculative at this point. Although large-vessel reopening occurs within a relatively short period of time, it is probable that reperfusion at the microcirculatory level is uneven and variable over time. In this situation, myoglobin might be released from different areas within the occluded segment at different times, depending on when microvascular reperfusion actually
occurs. A number of studies that have examined experimental ischemia and reperfusion indicate that there can be considerable tissue injury associated with re- 

covation of blood flow to an injured or infarcted area. This additional injury could also produce ongoing myoglobin release. Cobb et al.\(^2\) examined effects of acute cellular injury on coronary flow in a region exposed to a 2 hr occlusion by injecting radiolabeled microspheres at varying times after reestablishment of flow. In the period immediately after reperfusion, a hyperemic response was evident in all areas of ischemic tissue, but was considerably attenuated in the endocardium (which was presumably the area of most severe tissue necrosis and cell swelling). Four hours after the onset of reperfusion, subepicardial flow had returned toward baseline, while subendocardial flow was only 10% to 20% of its preoclusion level.

These findings indicate that prolonged ischemia may initiate tissue responses that bring about further reductions in flow as reperfusion occurs. Frame et al.\(^3\) examined myocardial reperfusion injury by studying cardiac cell membrane integrity with radiolabeled fragments of antecardiac myosin antibody. In eight animals, serial injections of antibody fragment were made after the onset of reperfusion that followed a 1 hr period of arterial occlusion. In each animal, antibody binding after 45 min of reperfusion was increased beyond that observed immediately after reperfusion, suggesting ongoing and increasing damage to the cell membrane. These studies raise the possibility that reperfusion injury contributes to the observed continuing release of myoglobin after reperfusion.

In the present study we were unable to address the issue of whether reperfusion injury was confined to tissue already destined to become necrotic before reperfusion. The basis for the absence of a continuing difference in GCV-arterial CK activity in contrast to the case for myoglobin in the present study is also unclear at this time. Because arterial levels of CK remain elevated so much longer than those of myoglobin, small GCV-arterial differences may have been difficult to discern analytically. CK released after the initial phase of reperfusion could, theoretically, have been inactivated in situ before its release, although a recent preliminary report by Sato et al.\(^4\) presents evidence against this.\(^4\) Alternatively, CK could have entered the systemic circulation via lymph rather than coronary venous blood. Possibilities such as these will need to be addressed in future studies of the basis of persistent GCV-arterial myoglobin differences.

Our initial patient data confirm the findings in dogs. In the nine patients who underwent successful reperfu-

sion, a sharp myoglobin peak occurred less than 2 hr after reopening of the vessel. This was in contrast to the findings in the patient in whom reperfusion was not successful. In this patient there was only a gradual increase in myoglobin concentration, with no clear peak, and the pattern of myoglobin release was similar to that observed in other studies of patients with acute myocardial infarction in whom reperfusion was not attempted. In this setting peak [Mb] typically occurs within 4 to 12 hr after admission to the coronary care unit, or 6 to 12 hr after onset of symptoms.\(^15\)\(^\text{-}19\) A recent preliminary study that examined patterns of myoglobin release during acute myocardial infarction confirmed the presence of an early peak in [Mb] with successful coronary arterial reperfusion and contrasted this with the later peaks noted in patients who received standard medical therapy.\(^20\)

In addition to the early peaks in [Mb] seen with successful reperfusion, a second, and perhaps practically more important, feature is the consistently rapid rate of rise in [Mb] immediately after reperfusion. The time required for [Mb] to increase from 25% to 100% of its peak value (T\(_{25-100}\)) was calculated for all patients studied (figure 8). The nine patients who underwent successful reperfusion, whether by intracoronary streptokinase, intravenous streptokinase, or PTCA, had an average T\(_{25-100}\) of 48 ± 27(SD) min. This value is similar in magnitude to that obtained in the 11 animals in the present study, in which T\(_{25-100}\) averaged 23 ± 10(SD) min. The T\(_{25-100}\) values for patients in whom reperfusion was successful contrast with that in the patient in whom it was unsuccessful (T\(_{25-100}\) = 5.7 hr) and with those derived from previous studies in which

\begin{figure}
\centering
\includegraphics{figure8.png}
\caption{\(T_{25-100}\) in patients undergoing successful (left and middle) and the one patient undergoing unsuccessful reperfusion (right). The consistently low values of \(T_{25-100}\) in the former group of patients contrast with that in the latter patient and with the values in medically treated patients in other series in which reperfusion was not attempted (open circles).}
\end{figure}
detailed myoglobin concentration-time curves were obtained during myocardial infarctions associated with attempts at reperfusion.\textsuperscript{18, 19} These differences in $T_{25-100}$ values suggest that this index can distinguish between the two patient groups without overlap.

As noted previously by several laboratories, skeletal muscle and cardiac myoglobin are inseparable by currently available radioimmunoassays. Thus, plasma myoglobin can be elevated independently of cardiac damage when skeletal muscle is injured. We are not aware, however, of peaks related to damage of skeletal muscle that are as rapid and prominent as those produced by reperfusion.

A prominent elevation in CK activity has been reported to be associated with successful coronary arterial reperfusion in patients receiving thrombolytic agents for acute myocardial infarction.\textsuperscript{21} Vatner et al.\textsuperscript{22} found a more rapid appearance of CK and an earlier time to peak in dogs undergoing reperfusion at 1 and 3 hr after coronary artery occlusion (time to peak CK activity = 4.2 ± 0.4 and 6.8 ± 0.5 hr, respectively) than in similar animals that did not undergo reperfusion (time to peak = 11.4 ± 0.5 hr). Similar results have been noted in patients treated with thrombolytic agents.\textsuperscript{2, 4} Successful reperfusion in these studies has generally been associated with peaks in CK activity at average times of 13 to 16 hr (vs 20 to 26 hr for patients not undergoing successful reperfusion). However, the large coefficients of variation of 20% to 50% indicate considerable variability and overlap in these values among individual patients in the two groups. Recently it has been noted that individual CK isoenzymes comprise three isoforms based on differences in isoelectric point.\textsuperscript{23} Current reports suggest that isoform proportions can be used to separate cases of successful vs unsuccessful reperfusion in the first few hours after lytic therapy.\textsuperscript{24}

In summary, reperfusion of injured myocardium results in a rapid release of myoglobin into the blood stream. In both animals and humans undergoing reperfusion, a sharp peak in [Mb] occurs within 1 to 2 hr of vessel reopening. We conclude that myoglobin concentration-time curves are a relatively direct marker of protein entry into the blood stream after reperfusion, and that the myoglobin peaks associated with reperfusion offer a useful additional indicator of reopening of the vessel when revascularization is undertaken and a coronary arteriogram is not available.

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References

myocardial infarction and control of the efficiency of coronary thrombolysis by a new myoglobin latex test. Circulation 70 (suppl II): II-153, 1984 (abst)


Patterns of myoglobin release after reperfusion of injured myocardium.
A K Ellis, T Little, A R Zaki Masud and F J Klocke

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