LABORATORY INVESTIGATION

MYOCARDIAL INFARCTION

Limitation of myocardial infarct expansion by reperfusion independent of myocardial salvage

JUDITH S. HOCHMAN, M.D., AND HEARAN ChOO, B.S.

ABSTRACT Very early reperfusion after coronary occlusion can reduce infarct size and preserve left ventricular function. Whether later reperfusion is of benefit is unclear. We studied the effect of very early reperfusion with myocardial salvage and “late” reperfusion without myocardial salvage on infarct expansion and aneurysm formation. Sixty-eight rats underwent left coronary artery ligation and were randomized to 30 min reperfusion, 2 hr reperfusion, or permanent coronary artery ligation. The animals were killed and the hearts examined at 2 weeks. Thirty minute reperfusion reduced infarct size, extent of transmurality, and infarct expansion. Two hour reperfusion reduced neither infarct size nor transmurality but inhibited infarct expansion. The results of this study in the rat preparation suggest a beneficial effect of “late” reperfusion on infarct expansion independent of myocardial salvage. If these results are confirmed in human beings, the period after myocardial infarction during which a patient can be considered for thrombolytic therapy or reperfusion by percutaneous transluminal coronary angioplasty could be greatly prolonged.


EXTENSIVE INVESTIGATION in animals and more recently in human beings has shown that infarct size can be reduced by very early reperfusion after coronary occlusion and that resultant left ventricular function is accordingly improved. Reimer et al. in their classic work demonstrated that after coronary occlusion there is a time-dependent wavefront of necrosis from subendocardium to subepicardium. However, whether there is benefit to patients who undergo coronary artery reperfusion either via thrombolytic therapy or percutaneous transluminal coronary angioplasty (PTCA) is still controversial, particularly when reperfusion is attempted late (e.g., beyond 4 hr) after coronary artery occlusion. Not all studies have shown a beneficial effect on left ventricular function.

Infarct expansion — thinning, and dilation of the infarct zone — is a complication of myocardial infarction that correlates with an increase in mortality, hemodynamic deterioration, and left ventricular rupture and is the substrate for left ventricular aneurysm formation. Expansion is thought to result from both stretching and slippage of sheets of myofibrils. If untreated, functional expansion, e.g., reversible systolic and diastolic bulging of ischemic myocardium, probably results in permanent structural pathologic changes of infarct expansion. Factors known to correlate with infarct expansion are infarct size, transmurality, and use of certain anti-inflammatory agents. Some inhibition of infarct expansion has been shown by left ventricular unloading.

Reperfusion after coronary ligation has been shown to salvage subepicardial cells in a time-dependent fashion and to change the pattern of necrosis from coagulation to contraction band necrosis. The purpose of this study was twofold: (1) to determine whether reducing infarct size would, as expected, reduce expansion and, more significantly, (2) to determine whether reperfused infarcted myocardium of the same size as nonreperfused myocardium would expand less, perhaps because of altered tissue properties.

Methods

Left coronary artery ligation was performed by a method previously described. Female Sprague-Dawley rats weighing 200 to 250 g were anesthetized with methohexital sodium (Brevital) 35 mg/kg, and the left chest was opened in the fifth to sixth intercostal space. The animals underwent positive-pressure ventilation with 95% O₂ and 5% CO₂. The pericardium was incised and opened and a 6-0 silk suture was placed around...
the left main coronary artery and tied. The chest was closed with 4-0 silk.

For pilot studies, the chest was opened in different groups of rats at 15, 20, 25, 30, 45, 60, 90, and 120 min and the ligature around the left coronary artery was cut. The chests were closed and these animals were killed at 2 days. Based on these results, which showed a reduction of infarct size at 30 min but not at 2 hr, these two times were chosen for the experiment.

Sixty-eight rats underwent left coronary artery ligation, and five rats underwent a sham operation in which a suture was placed around the left coronary artery but not tied. Twenty rats were reanesthetized at 30 min after coronary artery ligation and the suture was cut and the chest reclosed. Twenty-five rats were reanesthetized at 2 hr and the suture was cut and the chest reclosed. Twelve rats in the permanent ligation group had their chests opened and reclosed at 30 min and eleven had the same sham procedure at 2 hr. The ligature around the left coronary artery was left intact. Thus four groups of rat hearts were studied: (1) 30 min reperfusion, (2) 2 hr reperfusion, (3) permanently ligated coronary artery, and (4) sham.

All rats were maintained on standard rat chow and were housed two rats per cage. The animals were killed at 2 weeks because the prevalence and extent of infarct expansion has previously been shown to plateau by 1 week. The hearts were excised after methohexital sodium anesthesia, and the excised beating heart was submerged in cold 30 mM KCl to achieve diastolic arrest.

The hearts were perfused with 10% buffered formalin for 30 min through a cannula positioned in the aorta after the mitral valve was cut. The hearts were then further fixed with 10% formalin for at least 24 hr and were sectioned with a straight-edged blade from apex to base in 2-mm slices. Sections were prepared for histologic study and stained with hematoxylin and eosin. The microscopic slides were reviewed, magnified at 10 ×, and traced, with shading of the infarct zone. Microscopic slides were assessed for the degree of residual subendocardial and subepicardial cells in the infarct zone, hemorrhage, presence and extent of sequestered dead myofibrils, contraction band necrosis, granulation tissue, and fibrosis.

Data collection
Infarct size. Infarct size was calculated in two ways in order to assess possible differences in the experimental groups in the degree of hypertrophy of residual uninfarcted myocardium and differences in the degree of resorption of infarcted tissue:

\[
\frac{\text{Infarct mass}}{\text{Total LV mass}} \times 100\% \quad (1)
\]

\[
\left(1 - \frac{\text{Noninfarcted LV mass}}{\text{Normal LV mass}}\right) \times 100\% \quad (2)
\]

where normal LV mass is derived from uninfarcted hearts.

Extent of transmurality. Two formulas were used to calculate the extent of transmurality (%TM). The first formula assessed the degree to which the infarct extended completely through the left ventricular wall to touch the epicardium, and the second formula measured the percent of the total area subtended by the infarct's most lateral margins that was actually infarcted:

\[
\frac{\text{Infarct length touching epicardium}}{\text{Total infarct length}} \quad (3)
\]

The numerators were measured in each section and summed and were divided by the sum of the measurement of the denominator from all sections.

Aneurysm assessment. Hearts were graded as having aneurysmal dilation if the infarct formed a discrete bulge distorting the normal outer contour of the heart.

Expansion grading. The degree of expansion was graded based on the degree of thinning and dilation of the infarct zone by a previously reported qualitative method: 1+ = mild thinning, 2+ = mild thinning and dilation, 3+ = moderate thinning and dilation, 4+ = severe thinning and dilation.

Expansion index. To quantitate both the degree of left ventricular dilation and the degree of infarct wall thinning, the following formula was devised:

\[
\text{Expansion index} = \frac{\text{LV cavity area}}{\text{Total heart area}} \times \frac{\text{Infarcted septum thickness}}{\text{Infarcted LV free wall thickness}}
\]

Wall thicknesses were measured at the midpoints of the infarct and the septum. The section with the thinnest infarct and most marked cavity dilation was used for each heart. Total heart area is the cross-sectional area of the heart with right and left ventricles included; this ratio of dilation was used to correct for variations in the animal and heart sizes.

Statistics. Infarct sizes, extent of transmurality, and expansion index were compared by Student's t test. Distribution of expansion grades, aneurysm formation, and mortality rates were analyzed by the chi-square method. Values are expressed as mean ± SEM.

Results
Pilot study. Rat hearts with coronary artery occlusion for 30 min or less had smaller infarcts (figure 1, A, see below), and these hearts, when examined microscopically at 2 days, had more hemorrhage and contraction band necrosis than hearts with permanently ligated coronary arteries examined at 2 days. Two hour reperfusion did not reduce myocardial infarct size. These hearts, examined at 2 days, had more hemorrhage and contraction band necrosis than permanently ligated ones.

Thirty minutes and 2 hr were therefore chosen for the main experiment, the former as an example of myocardial salvage through reperfusion and the latter as an example of "late" reperfusion without myocardial salvage.

Operative mortality. Of 20 rats undergoing reperfusion at 30 min, three died, two postoperatively and one at 3 days. Four of 25 rats in the 2 hr reperfusion group died postoperatively. Two of 23 rats in the permanently ligated group died postoperatively. No animal in the sham-operated group died. These mortality rates were not significantly different.

Histology. The findings of the pilot study of 2 day infarcts are noted above. The morphology of the 2 week infarcts was somewhat different for reperfused...
Llaboratory Investigation—Myocardial Infarction

compared with permanently ligated hearts. All infarcts had some granulation tissue, fibroblasts, and loose fibrous tissue and exhibited an accelerated healing phase compared with humans as has been described.\textsuperscript{19,32} The presence and extent of hemorrhage at 2 weeks was similar for all groups. The 30 min reperfused group had smaller, extensively nontransmural infarcts (figure 1) with large areas of residual subepicardial and subendocardial cells.

Histologically, on careful examination of the four or five sections per heart, there were slightly more hearts with islands of residual subepicardial cells in the 2 hr reperfused group than in the permanently ligated group (13/21 vs 7/21, $\chi^2 = 3.6$, $p < .1$). Quantitatively, when measured from 10-fold magnified tracings, no differences were seen in extent of transmurality and infarct size (figure 1).

Only one reperfused heart had sequestered dead myofibrils in the center of the infarct at 2 weeks compared with 12 of 21 in the group with permanently ligated coronary arteries, suggesting that reperfused infarcts healed faster (0/17 30 min reperfused vs 12/21 permanently ligated, $\chi^2 = 16$, $p < .005$; 1/21 2 hr reperfused vs 12/21 permanently ligated, $\chi^2 = 13.5$, $p < .005$).

**Infarct size.** Infarct size was calculated by two methods as previously described\textsuperscript{19,33} because the first method might underestimate or overestimate size due to variations in tissue resorption, hemorrhage, and edema, and the second would be affected by hypertrophy of residual uninfarcted myocardium. Statistical differences may be found if either of these processes were different between the study groups. Within each of the two groups (30 min reperfused, 2 hr reperfused, and permanently ligated), these two methods yielded similar infarct sizes (table 1).

Comparing infarct size in the three groups demonstrated that 30 min reperfused infarcts were significantly smaller (17 ± 1.9% left ventricle) than either 2 hr reperfused (26 ± 2.4% left ventricle) or permanently ligated hearts (27 ± 2.4% left ventricle) (figure 1, A; $p < .001$). There was no difference in infarct size between 2 hr reperfused and permanently ligated groups ($p = NS$).

**Transmurality.** Extent of transmurality, or the degree to which the infarct involves the full thickness of the left ventricular wall, may be important in determining

![](image.png)

**FIGURE 1.** Infarct size (A), extent of transmurality (B), and expansion index (C) for 30 min reperfused, 2 hr reperfused, and permanently ligated hearts (mean ± SEM).

**TABLE 1**

<table>
<thead>
<tr>
<th>Infarct size</th>
<th>Group 1 (n = 17)</th>
<th>Group 2 (n = 21)</th>
<th>Group 3 (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 min</td>
<td>16.8 ± 1.9</td>
<td>26.1 ± 2.4</td>
<td>27 ± 2.4</td>
</tr>
<tr>
<td>2 hr reperfused</td>
<td>12.6 ± 3.2</td>
<td>22 ± 5.2</td>
<td>26 ± 4.2</td>
</tr>
</tbody>
</table>

Infarct sizes calculated by two methods are shown for 30 min reperfusion, 2 hr reperfusion, and permanently ligated hearts (percent left ventricle ± SEM). Formulas 1 and 2 are shown in the Methods section. Group 1 vs 2, $p < .01$; group 2 vs 3, $p = NS$; group 1 vs 3, $p < .02$; formula 1 vs 2 within each group, $p = NS$.  

Vol. 75, No. 1, January 1987

301
TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 17)</td>
<td>(n = 21)</td>
<td>(n = 21)</td>
</tr>
<tr>
<td>30 min reperfused</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formula 3</td>
<td>27 ± 6.5</td>
<td>69 ± 3.3</td>
<td>64 ± 5.2</td>
</tr>
<tr>
<td>Formula 4</td>
<td>52 ± 1.9</td>
<td>73 ± 2.2</td>
<td>74 ± 2.6</td>
</tr>
</tbody>
</table>

The extent of transmurality (% TM) was calculated by formulas 3 and 4 (see Methods).

Group 1 vs 2, p < .001; group 1 vs 3, p < .001; group 2 vs 3, p = NS.

The degree of expansion. Therefore we used the two formulas noted above that assessed (1) the degree to which the infarct was fully transmural (e.g., extended to touch the epicardium) and (2) the percent of the total area subtended by the infarct that was actually infarcted. There was a significant reduction in the degree of transmurality of 30 min reperfused infarcts compared with permanently ligated infarcts (27 ± 6.5% vs 64 ± 5.2%; p < .001) (table 2 and figure 1, B). There was also a reduction in the percent transmurality of 30 min reperfused compared with 2 hr reperfused hearts (27 ± 6.5% vs 69 ± 3.3%; p < .001). These differences were seen by both methods of calculation.

Both 2 hr reperfused and permanently ligated hearts had extensively transmural infarcts as measured by both methods, and there was no difference between these groups.

Expansion. There was a significant inhibition of expansion by reperfusion as demonstrated by a shift to lower expansion grades in hearts that underwent either 30 min or 2 hr reperfusion compared with permanently ligated hearts (p < .01) (figure 2). Examples of different expansion grades with reperfusion time are shown in figure 3.

To further quantitate the dilation and infarct wall thinning that characterize expansion, we developed the method described above. Higher expansion grades significantly correlated with higher expansion indexes (r = .72, p < .001).

Figure 1 compares myocardial infarction size, percent transmurality, and expansion index for the three groups and shows that 30 min reperfused infarcts, which were smaller and less extensively transmural, underwent less marked expansion than 2 hr reperfused and permanently ligated infarcts. Despite the fact that 2 hr reperfused infarcts were smaller and no less transmural than permanently ligated infarcts, expansion was significantly inhibited as demonstrated by smaller expansion indexes.

**Aneurysm formation.** Significantly fewer aneurysms (figure 4) formed in the reperfused compared with the nonreperfused groups (2/42 vs 7/21; p < .005). There were no aneurysms in the 30 min reperfused group. The number of aneurysms in the 2 hr reperfused hearts

**FIGURE 2.** Distribution of expansion grades for 30 min reperfused (A), 2 hr reperfused (B), and permanently ligated (C) hearts. (p < .01 for 30 min and 2 hr compared with permanently ligated).
was less than that in the permanently ligated group but did not quite achieve statistical significance (2/22 vs 7/21; p = .053).

Discussion

Evidence is accumulating that very early reperfusion in human beings as well as in animal preparations may reduce infarct size, improve left ventricular function,1-4 and reduce mortality after myocardial infarction.34-36 The results of this study in the rat preparation support the hypothesis that later reperfusion, with or without a reduction in infarct size, inhibits aneurysm formation and infarct expansion.

Infarct expansion has been shown to develop early after infarction, to progress in severity days after infarction,12, 14, 15 and to be followed by late remodeling with dilation and hypertrophy of remote uninfarced myocardium. Very early systolic bulging is functional expansion undergone by ischemic tissue and is potentially reversible.37-39 Expansion of necrotic myocardium is probably caused by both slippage of sheets of myocardial cells and stretching of myofibrils,18 and its potential reversibility is not known. Expansion correlates with an increased mortality after myocardial infarction and is associated with an increase in both

**FIGURE 4.** Distribution of hearts with and without aneurysmal dilation in the reperfused vs permanently ligated groups.

<table>
<thead>
<tr>
<th>Aneurysm</th>
<th>Reperfused</th>
<th>Permanently ligated</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Aneurysm</td>
<td>36</td>
<td>14</td>
</tr>
<tr>
<td>Aneurysm</td>
<td>2</td>
<td>7</td>
</tr>
</tbody>
</table>

P < 0.005
sudden death\textsuperscript{13} and congestive heart failure.\textsuperscript{12, 14} Expansion is also a substrate for left ventricular rupture\textsuperscript{15} and aneurysm formation.\textsuperscript{16, 17} Preventing its occurrence should logically prevent its adverse sequelae.

This study used an established rat preparation of infarction known to produce extensively transmural myocardial infarctions after permanent coronary artery ligation; these infarcts best resemble the type of human infarcts that expand.\textsuperscript{11, 40} The time course for examination of the myocardial infarction was one at which a plateau in the prevalence and severity of expansion has been demonstrated.\textsuperscript{16, 19} The time course of ischemic injury and healing of infarcts in the rat is accelerated compared with that in human beings.

Because measurements of the degree of expansion are important in assessing differences between groups, expansion was graded quantitatively as well as by an established qualitative method.\textsuperscript{19} A single index was devised that measures degree of cavity dilation, corrects for variation in heart size, and measures degree of infarcted wall thinning.

The results showed that when infarct size is reduced by 30 min reperfusion, extent of transmurality is reduced and the incidence and degree of expansion are reduced. This is an expected finding, given that larger infarct size and transmurality are known to predispose to more expansion,\textsuperscript{19, 20, 41} and confirms that reducing infarct size within the risk region does reduce expansion. More significantly, the 2 hr reperfused rat hearts had the same infarct size and extent of transmurality as the permanently ligated hearts and yet had less severe expansion.

The mechanism by which infarct expansion and aneurysm formation was reduced with reperfusion but without myocardial salvage is unknown. The pilot study and others\textsuperscript{42-45} have shown increased hemorrhage, cell swelling, edema, and contraction band necrosis\textsuperscript{27} in reperfused infarcts when examined pathologically early after infarction, and it may be that these factors make the infarcted wall stiffer and more able to resist systolic bulging. The hypothesis that early edema may be protective against expansion is suggested by studies showing detrimental effects of early indomethacin and ibuprofen on expansion.\textsuperscript{22, 24}

Although scar stiffness was recently demonstrated to be the same in reperfused and nonreperfused myocardial infarctions in rabbits,\textsuperscript{46} the times after myocardial infarction examined in that study were 1 to 3 weeks. These investigators did not look at days 1 to 5 when expansion is occurring and progressing in rats.

Extent of transmurality was calculated in two ways to assess both salvage of the subepicardial rim and the percent of the left ventricular wall thickness that was infarcted. No difference between the late reperfused (2 hr) and permanently ligated groups were found by either method, suggesting that reduction in the degree of transmurality is not the mechanism by which expansion is inhibited in these “late” reperfused infarcts. On histologic examination, there was a trend toward more hearts containing islands of residual subepicardial cells in the 2 hr reperfused group compared with the permanently ligated group, but this was not quantitatively important and not statistically different. This has been postulated as a mechanism of inhibition of expansion\textsuperscript{47} in another study. It is possible that the limits of the technique of examining four to five cross sections per heart may have missed viable myocardial cells, and if more were missed in the 2 hr reperfused hearts differences in the degree of transmurality may have been missed.

A third mechanism by which expansion may be inhibited without myocardial salvage is that the rate of healing of the infarct is accelerated. This is suggested in this study by the fact that fewer reperfused infarcts had residual sequestered dead myofibrils at 2 weeks than permanently ligated hearts. Accelerated healing of reperfused infarcts in pigs and inhibition of aneurysm formation has been reported previously.\textsuperscript{48}

Although 30 min reperfusion totally inhibited expansion in greater than 50% of the cases, 2 hr reperfusion mainly reduced the severity of expansion. Prior work in this preparation has shown that although expansion occurs early (day 1) before an inflammatory reaction is seen, it progresses in severity in the first 5 to 7 days in the rat as resorption of dead myofibrils occurs.\textsuperscript{19} This second phase of progressive expansion may be inhibited by accelerated healing after reperfusion, resulting in less severe expansion.

If these findings of inhibition of infarct expansion and aneurysm formation with reperfusion at a time later than that allowing for salvage of ischemic myocardium are corroborated in human beings, there are significant implications for the timing and consideration of reperfusion in humans. If inhibiting expansion prevents its adverse sequelae, then the reperfusion modalities of thrombolytic therapy, PTCA, or coronary bypass grafting at later times after the onset of myocardial infarction, when infarct size may not be reduced (e.g., 4 to 24 hr), should be carefully examined with regard to left ventricular shape changes and function in human beings. More experimental work is necessary to find the latest time after myocardial infarction that this beneficial effect can be seen in animals and whether it is demonstrable in human beings.
We thank Esther Yulfo for her excellent assistance in the preparation of this manuscript.

References


34. GISSI: Effectiveness of intravenous thrombolytic treatment in acute myocardial infarction. Lancet 1: 397, 1986


42. Roberts CS, Schoen FJ, Kloner RA: Effect of coronary reperfusion on myocardial hemorrhage and infarct healing. Am J Cardiol 52: 610, 1983


Limitation of myocardial infarct expansion by reperfusion independent of myocardial salvage.

J S Hochman and H Choo

Circulation. 1987;75:299-306
doi: 10.1161/01.CIR.75.1.299

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1987 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/75/1/299

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/