Editorial Comment

Does Reperfusion Induce Myocardial Necrosis?

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Reperfusion Injury

While coronary thrombolytic therapy in clinical practice is well established for acute myocardial infarction, the injury associated with reperfusion (“reperfusion injury”) is still under intense investigation regarding its nature and the strategy for its prevention. Reperfusion injury has been defined as cell injury caused by reperfusion itself, in contradistinction to cell injury caused by the preceding ischemia. It refers to cell necrosis or is used in a broader way to include mechanical dysfunction (myocardial stunning), arrhythmia (reperfusion arrhythmia) and vascular damage (no-reflow phenomenon). In contrast to myocardial stunning, reperfusion arrhythmia and no-reflow phenomenon, the existence of reperfusion-induced lethal myocardial injury is still highly controversial.

Approaches to Detect Reperfusion-Induced Myocardial Necrosis

Does reperfusion itself cause myocardial necrosis? There have been two approaches to this question. The first is to test the effect of pharmacological agents, which are administered during reperfusion, on myocardial infarct size. If the myocardial infarct size is reduced by an agent given only at the time of reperfusion, it indirectly demonstrates the presence of reperfusion-induced myocardial necrosis that is diminished by that agent. Of numerous agents tested for this purpose, superoxide dismutase (SOD) is the agent most extensively examined over the past 6 years. In our series of experiments, SOD coadministered with catalase for a short peri-reperfusion period delayed myocardial necrosis; however, it did not limit the ultimate myocardial infarct size in the rabbit. Furthermore, SOD failed to alter the infarct size regardless of the duration of coronary occlusion, suggesting that a “window of ischemia duration,” within which oxygen free radicals upon reperfusion substantially contribute to cell death, is unlikely to be present. Our findings corroborated several other studies that determined infarct size histologically at 2–4 days after ischemia. On the other hand, there are also studies reporting infarct size limitation by SOD. A clear explanation is not available for the conflicting results in laboratory experiments as recently detailed in extensive reviews by Engler and Gilpin and also Kloner et al in this journal.

An alternative approach to lethal reperfusion injury, which was employed by Ganz and colleagues in this issue of Circulation and previously by Hofmann et al, is to determine the difference in the size of nonreperfused and reperfused infarcts after the same length of ischemia. Although this approach would be able to screen the reperfusion-induced myocardial necrosis regardless of its mechanism, two requirements must be met in the experiment to make this approach valid. First, the independent determinants of infarct size should be comparable between the reperfused and nonreperfused infarcts. Second, the method of infarct determination must have the same accuracy for both nonreperfused and reperfused infarcts. Although major determinants of infarct size are known and measurable, the accuracy of infarct sizing is not necessarily easy to ensure because of limitations in the methods to differentiate dead and viable myocytes. There are three techniques that have been frequently employed to delineate experimental myocardial infarction: light microscopy, electron microscopy, and tetrazolium stainings for macrohistochemistry. Hematoxylin-eosin staining for light microscopy has been the gold standard method for estimation of infarct size; however, it does not differentiate infarct until 6–24 hours after ischemia. Although electron microscopy is probably the most sensitive technique to detect myocyte necrosis, it is practically impossible to delineate the whole infarct by this method. Tetrazolium staining with triphenyltetrazolium chloride (TTC) or nitro blue tetrazolium (NBT) has been a popular technique because it is easy to perform and permits early identification of infarcts. However, the conditions under which tetrazolium staining accurately delineates myocardial infarct needs to be reconsidered.
Tetrazolium Staining

It has been explained that viable myocardium reduces tetrazolium to formazan pigments by diaphorases that use NADH or NADPH as electron donors. Infarcts are identified as tetrazolium staining defects, which are attributed to loss of cofactors and/or dehydrogenases and diaphorases in necrotic myocardium. Thus, the factors influencing the washout process of cofactors and enzymes presumably modify the degree of tetrazolium staining in the infarcted myocardium. Actually, previous studies have suggested that the accuracy of tetrazolium staining depends on the time after the onset of ischemia, reperfusion, and duration of coronary occlusion.

There has been good correlation in infarct sizing between hematoxylin-eosin histology and tetrazolium staining in the canine and rat models 6 hours after permanent coronary occlusion. However, when tetrazolium staining was applied at 3 hours after coronary occlusion, infarction was not identified in some of the animals.

Because reperfusion accelerates the destruction of myocardial ultrastructures and washout of enzymes, tetrazolium staining should detect myocardial infarcts sooner if the ischemic myocardium is reperfused. How long is reperfusion necessary for reliable diagnosis of infarct by tetrazolium staining? Schaper et al reported that myocardial infarct size (NBT staining) was similar in dogs that received 90-minute reperfusion and 48-hour reperfusion. In a study using the rabbit model subjected to 45-minute coronary occlusion, Shirato et al observed that infarct size (TTC staining) was not different in the 3-hour and 24-hour reperfusion groups. Furthermore, both histology and TTC staining in the same hearts gave good agreement in infarct size 24 hours after ischemia/reperfusion. Thus, 3-hour reperfusion in the rabbit and 90-minute reperfusion in the canine heart are probably long enough for reliable infarct sizing by tetrazolium staining. Whether shorter reperfusion of infarct always permits accurate infarct identification by tetrazolium staining is unknown.

The actual size of infarct is another possible determinant of accuracy of tetrazolium staining. Recently, Horneffer et al provided evidence that even at 48 hours after reflow, NBT staining may markedly underestimate small infarcts. After 15 minutes of coronary occlusion in the pig heart, infarct size was 1.2% of area at risk by NBT versus 10.9% by histology, and after 30 minutes of occlusion, infarct size was 28.6% by NBT versus 56.1% by histology. When the ischemic period was extended to 90 minutes or longer, there was better correlation in infarct sizing by the two methods. They proposed that underestimation of small infarcts by NBT may be due to overshadowing patchy scattered foci of necrosis by the viable stained myocardium.

The Present Study

In the study by Ganz and colleagues, two myocardial regions were subjected to ischemia of varying lengths (90–240 minutes) in a single heart. One of the ischemic regions was reperfused for 5 minutes whereas the other region received an additional 5-minute ischemia. TTC staining showed no difference in infarct sizes between the nonreperfused and reperfused regions. Because TTC staining was applied to very early ischemia/reperfusion, electron microscopy was used to confirm the validity of TTC staining. Their data thus suggest that the extension of myocardial necrosis by reperfusion per se is unlikely in the dog heart. Those results were consistent with earlier findings by Hofmann et al that myocardial infarct size after 3- or 6-hour ischemia did not differ between dogs with or without 60- or 90-minute reperfusion.

In the present study by Ganz et al unfortunately the two ischemic regions were not completely comparable regarding determinants of myocardial infarct size. Duration of ischemia was longer by 5 minutes in the nonreperfused region than the reperfused region. In addition, the subepicardial collateral flow tended to be higher in the nonreperfused region than the reperfused region although the difference in collateral flow did not reach the statistically significant level. The authors showed in their preliminary study that this 5-minute difference in ischemia duration did not result in discernible extension of infarct in their model. This finding supports the validity of their model and, at the same time, reveals its limitations for detecting reperfusion-induced myocardial necrosis. Since the wave front of myocardial infarct progresses toward the subepicardial regions during the first 6 hours after coronary artery occlusion in the dog heart, once the myocardial infarct starts developing there should have been some extension of infarct during that additional 5 minutes. Thus, the failure to detect infarct extension during that 5-minute ischemia in the preliminary experiment suggests that their model would not be able to detect the reperfusion-induced myocardial necrosis if its mass were equivalent to or smaller than that which went undetected in the 5-minute progression of the infarct wave front. Nevertheless, the data of Ganz et al do not support the concept that a substantial proportion of the infarct following ischemia/reperfusion occurs upon reperfusion.

There are possibilities, however, that a significant mass of salvaged myocardium is irreversibly injured later than 5 minutes after reperfusion, possibly by infiltrating leukocytes and that reperfusion-induced myocardial necrosis is dominant following shorter ischemia than that examined by Ganz et al.

Conclusion

Myocardial necrosis caused by reperfusion per se is methodologically difficult to prove or disprove. A consensus has not been obtained on whether free
radical mediated–lethal reperfusion injury exists. The present study by Ganz et al.\(^2\) contradicts the hypothesis that a substantial quantity of myocardium is irreversibly injured upon reperfusion after 90–240 minutes of ischemia. However, the possibility still remains that lethal reperfusion injury occurs during later reperfusion or following shorter and less severe ischemia.

**References**


6. Uraizee A, Reimer KA, Murry CE, Jennings RB: Failure of superoxide dismutase to limit size of myocardial infarction after 40 minutes of ischemia and 4 days of reperfusion in dogs. Circulation 1987;75:1237–1248


25. Reimer KA, Jennings RB: The waveform phenomenon of myocardial ischemic cell death: II. Transmural progression of necrosis within the framework of ischemic bed size (myocardial at risk) and collateral flow. Lab Invest 1979;40:633–644

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_Circulation_. 1990;82:1070-1072
doi: 10.1161/01.CIR.82.3.1070

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1990 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

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