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Lethal Myocardial Reperfusion Injury: Fact or Fiction?

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The objective of this editorial is to address three specific questions regarding lethal myocardial "reperfusion injury":

1. Does lethal reperfusion injury exist?
2. Is lethal reperfusion injury an important concern from a clinical standpoint?
3. Should treatments and pharmacologic interventions be designed and marketed to attenuate lethal reperfusion injury?

Does Lethal Reperfusion Injury Exist?

Many investigators have questioned the existence of "lethal reperfusion injury," that is, the ability of reperfusion to cause the death of cells that are viable before the moment of reperfusion, and have argued that reperfusion merely accelerates the histologic expression of necrosis. The experimental studies that have been performed to prove or disprove the existence of lethal reperfusion injury have yielded conflicting data. Dr. Ganz and colleagues have published histologic observations that are claimed to disprove the existence of lethal reperfusion injury [1]. They compared the extent of necrosis in the reperfused and nonreperfused halves of a single ischemic territory and concluded that reperfusion did not extend the boundary of necrosis.

Gottlieb et al. [2] examined the effects of reperfusion on the appearance of nucleosomal ladders of DNA fragments, the hallmark of apoptosis. Typical signs of apoptosis were observed in the myocardium of all rabbits subjected to 30 minutes of coronary artery occlusion followed by 4 hours of reperfusion. Nucleosomal ladders were not observed in ischemic myocardium after either 30 minutes or 4.5 hours of ischemia without reperfusion. Thus the hallmark of apoptosis was detected only in myocardium subjected to reperfusion after ischemia, supporting the notion that reperfusion causes late cell death of cardiac myocytes.

Pharmacologic studies also have yielded conflicting data regarding the existence of lethal reperfusion injury. Possible explanations for the conflicting data obtained by different laboratories include the use of different species, for example, rats, cats, dogs, pigs, and primates; differences in the dose and duration of drug treatment; differences in the durations of coronary artery occlusion and reperfusion; different techniques for measuring the extent of myocardial infarction; and failure to measure covariates of infarct size, for example, collateral blood flow. Each of the following agents, however, has been demonstrated to limit the extent of myocardial injury, even when the treatment was initiated just before myocardial reperfusion, infarct size was measured at least 24 hours after reperfusion, and infarct size was adjusted for differences in collateral blood flow (Table 1). Superoxide dismutase conjugated to polyethylene glycol (PEG-SOD), an enzymatic "scavenger" of superoxide anions; N-(2-mercaptopropionyl)-glycine (MPG), a low molecular-weight antioxidant; poloxamer 188, a nonionic surfactant; and agents directed against adhesion molecules that mediate leukocyte-endothelial interactions, for example, $F(ab')_2$ fragments of a monoclonal antibody directed against Mo-1(CD11b/CD18). Nevertheless, such "positive" studies that demonstrated reductions of infarct size have been dismissed by critics who have offered the following explanations for the smaller infarct sizes of the "treatment" groups compared with the control groups; higher collateral blood flow in the drug-treated animals compared with the control animals, an artifact of the method of measuring infarct size, and a delayed development of necrosis rather than a permanent salvage of ischemic myocardium. Despite the conflicting experimental findings and the ensuing controversy, clinical studies have been conducted to evaluate several agents that are purported to attenuate reperfusion injury.

Is Lethal Reperfusion Injury an Important Concern from a Clinical Standpoint?

Recently published data have provided evidence that reperfusion of ischemic myocardium in patients with acute myocordial infarction (MI) is accompanied by myocardial release of cytokines and free radicals, and

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Table 1. Agents that limit reperfusion injury

Drug	Drug started	Duration of ischemia (min)	Duration of reperfusion (days)	Control I/AR	Drug I/AR	p (ANCOVA)[a]	Ref.
PEG-SOD	15 min pre-reperfusion	90	4	44%	29%	0.017	A
MPG	15 min pre-reperfusion	90	2	58%	29%	<0.001	B
MPG	30 min post-reperfusion	90	2	58%	29%	<0.001	B
Anti-Mol $F(ab')_2$	45 min pre-reperfusion	90	3	37%	22%	0.008	C
Poloxamer 188	15 min pre-reperfusion	90	3	43%	25%	0.002	D

[a]p for analysis of covariance using I/AR (Infarct/area at risk) as dependent variable and collateral blood flow as independent variable.
Reference A: Tamura et al. *Circ Res* 1988;63:944–959.
Reference B: Horwitz et al. *Circulation* 1994;89:1792–1801.
Reference C: Simpson et al. *Circulation* 1990;81:226–237.
Reference D: Schaer et al. *Circulation* 1994;90:2964–2975.

surface expression on neutrophils of the B_2-integrin Mac-1 and L-selectin [3,4]. Although these findings are consistent with the activation of neutrophils and production of free radicals, they do not prove that reperfusion causes lethal myocardial injury. It has been suggested that the administration of thrombolytic therapy is associated with an increase in mortality during the first 24 hours. Even if there is an excess of early deaths that are caused by reperfusion injury, overall survival after acute myocardial infarction has been improved substantially by the use of thrombolytic therapy. Also, left ventricular function is often preserved in patients with acute MI who receive thrombolytic therapy or undergo primary coronary angioplasty (PTCA). One week after acute MI, the mean left ventricular ejection fraction was >50% among the control patients enrolled in the superoxide dismutase (SOD) trial [5]. Thus, a prohibitively large sample size may be required to demonstrate either a meaningful improvement in left ventricular function or a reduction in mortality by treatment with an agent that limits reperfusion injury.

The GUSTO angiographic substudy demonstrated that "incomplete" reperfusion does not improve survival after acute MI. Establishment of TIMI grade 3 flow reduces mortality, while patients with TIMI grade 2 flow after thrombolysis have the same mortality as patients with TIMI grade 0. One possible explanation of TIMI 2 flow after PTCA or thrombolytic therapy is the no-reflow phenomenon due to microvascular injury. Experimental studies have indicated that the coronary microcirculation may be injured by leukocytes that adhere to damaged endothelium. Agents that inhibit leukocytes or their cytotoxic products may protect the microcirculation and prevent no reflow. Thus, lethal reperfusion injury may be an important clinical concern if the no-reflow phenomenon is a manifestation of lethal reperfusion injury, and if prevention of no reflow can increase the percentage of patients who achieve TIMI 3 flow after PTCA or thrombolytic therapy. Contrast echocardiography might be a useful tool to evaluate the effect of therapies on no reflow after acute MI.

Should Treatments and Pharmacologic Interventions Be Designed and Marketed to Attenuate Lethal Reperfusion Injury?

The reperfusion injury hypothesis has been tested in several clinical trials that have been published or presented at scientific meetings. Human recombinant SOD was studied in a multicenter, placebo-controlled randomized trial of 120 patients with acute MI undergoing coronary angioplasty [5]. Neither regional nor global left ventricular function was improved by treatment with SOD.

A perfluorochemical, Fluosol, and a component of Fluosol, RheothRx, have been demonstrated to inhibit leukocyte migration and to limit infarct size in animal models of MI. TAMI-9 was a large randomized study of Fluosol in patients with acute MI who were treated with tPA [6]. Four hundred and thirty patients were randomized in a prospective, open-label study to receive either 15 ml/kg of Fluosol intravenously, or no Fluosol. Treatment with Fluosol was not associated with improvement in ventricular systolic function, reduction in infarct size measured by thallium scintigraphy, or clinical outcome. RheothRx (Poloxamer 188, N.F.), a nonionic surfactant that also has been shown to inhibit leukocyte migration and to limit infarct size in animal models of MI, also has been tested in clinical trials [7,8]. In a multicenter study of patients receiving thrombolytic therapy for acute MI, 114 patients underwent a 2:1 randomization to intravenous RheothRx or placebo [8]. The patients treated with RheothRx had smaller infarcts measured by Tc-99m-sestamibi imaging, higher left ventricular ejection fraction at the time of hospital discharge, and fewer major clinical events (death, reinfarction, or

shock). Two subsequent studies of RheothRx obtained negative results. In a trial of 150 patients undergoing primary PTCA, treatment with RheothRx did not improve ejection fraction or infarct size [7].

At least two ongoing clinical trials will provide additional data to "fuel" the reperfusion injury controversy. CALYPSO is a double-blind, placebo-controlled, multicenter study of CY 1503 in patients with acute MI treated with primary PTCA. CY 1503 is a compound that blocks neutrophil interaction with E-selectin and P-selectin [9]. Experimental studies have demonstrated that administration of CY 1503 just before reperfusion reduces canine myocardial infarct size and neutrophil infiltration after coronary artery occlusion for 90 minutes followed by reperfusion [9]. The CALYPSO study will use technetium-99m sestamibi imaging to quantitate the effect of CY 1503 on infarct size measured 5–7 days after PTCA for acute MI.

LIFT is a placebo-controlled, multicenter study of TLC C-53 as an adjunct to thrombolytic therapy in patients with acute MI. TLC C-53 is a liposomal PGE_1 that has been shown to limit canine infarct size when the agent is administered by intravenous bolus immediately before myocardial reperfusion [10].

Most of the experimental studies that demonstrated a reduction of infarct size employed an ischemic period of between 1 and 2 hours. There is scant evidence that any agent will salvage ischemic myocardium after more than 2 hours of ischemia. The average delay between symptom onset and treatment was 4 hours in the clinical trials of SOD [5] and Fluosol [6]. Therefore, the CALYPSO and LIFT studies may not demonstrate a beneficial effect on infarct size unless the time to treatment is more rapid. It will be interesting, however, to learn whether either therapy increases the proportion of patients who achieve TIMI 3 flow after reperfusion, thereby providing indirect evidence for the prevention of "no reflow."

As an investigator in the cardiovascular pharmacology laboratory, I became convinced that free radicals and/or leukocytes can cause lethal myocardial reperfusion injury. As an interventional cardiologist I have treated occasional patients with acute MI who develop increased ST-segment elevation and chest pain after primary PTCA. Also, I have had to "settle" for TIMI 2 flow despite successful dilatation of the coronary occlusion. Thus, I suspect that some patients may suffer reperfusion injury of the coronary microcirculation. As a clinical investigator I have remained hopeful that a precise method of measuring myocardial infarct size, for example, sestamibi imaging, may permit the detection of myocardial salvage by an agent that works in the animal laboratory. The results of the clinical trials that have been performed, however, have caused me to doubt that lethal myocardial reperfusion injury is an important concern in the majority of patients who undergo primary PTCA or thrombolytic therapy. Therefore, if both the CALYPSO and LIFT studies fail to reveal clinical benefits of treatment with CY 1503 or TLC C-53, it seems unwise for pharmaceutical companies or clinical investigators to invest resources in the development of therapies to prevent reperfusion injury.

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