

# Consequences of reperfusion

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## Abstract

Coronary artery reperfusion after temporary coronary occlusion is the only measure that can prevent irreversible damage of cardiac tissue. The later that reperfusion is established, the less myocardial tissue can be salvaged: in the dog, complete salvage can be achieved after brief episodes of ischemia of less than 20 min, whereas nearly complete necrosis of the area at risk occurs with reperfusion after more than 6 h. Consequences of reperfusion may manifest as progressive microvascular damage (“no reflow”) in infarcted myocardium and reversible contractile dysfunction (“stunning”) in salvaged cardiomyocytes. Very brief ischemic episodes followed by reperfusion can put the heart into a “preconditioning” state, a condition that increases myocardial tolerance towards a long ischemic insult.

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## Introduction

In most species, coronary artery occlusion results in myocardial ischemia because the collateral circulation is insufficient to maintain adequate supplies of oxygen and substrate [1]. In effect, coronary reperfusion is the only means of restoring aerobic metabolism and preventing irreversible damage of cardiac tissue. Therefore, the consequences of reperfusion – the most important of which will be discussed in this review – are always implicitly the consequences of the preceding period of temporary ischemia. Factors that potentially determine the final consequences of reperfusion, in a qualitative or quantitative manner, are: the duration and severity of ischemia, the volume of the ischemic area at risk, adjunctive therapeutic interventions before and during ischemia, and energetic demands during ischemia. Whether the specific mode of reperfusion and adjunctive therapy given during reperfusion can influence the ultimate volume of necrosis remain under investigation.

## Salvage of myocardium at risk

In the dog, proximal coronary artery occlusion for up to 20 min, followed by re-opening of the artery, results in reversible alterations to the myocardium; however, after more than 20 min, significant amounts of necrosis develop (*Figure 1*) [1–3]. In their landmark publication in 1977, Reimer et al [4] described a wavefront of myocardial necrosis progressing from the endocardial towards the epicardial surface of the canine heart as the duration of the coronary artery occlusion was increased. When the occluded circumflex coronary artery was re-opened after 40 min of ischemia, 38% of the myocardium at risk had become necrotic; after 3 h 57% developed necrosis, after 6 h 71% was necrotic, and after 24 h 85% became necrotic. In most experimental studies in the dog, reperfusion within a period of 6 h after the onset of ischemia was associated with significant subepicardial salvage, with variation depending upon the amount of collateral flow; after 6 h of coronary occlusion, nearly the entire area at risk was already necrotic [5,6]. However, if ischemia during coronary occlusion is more severe, for instance in

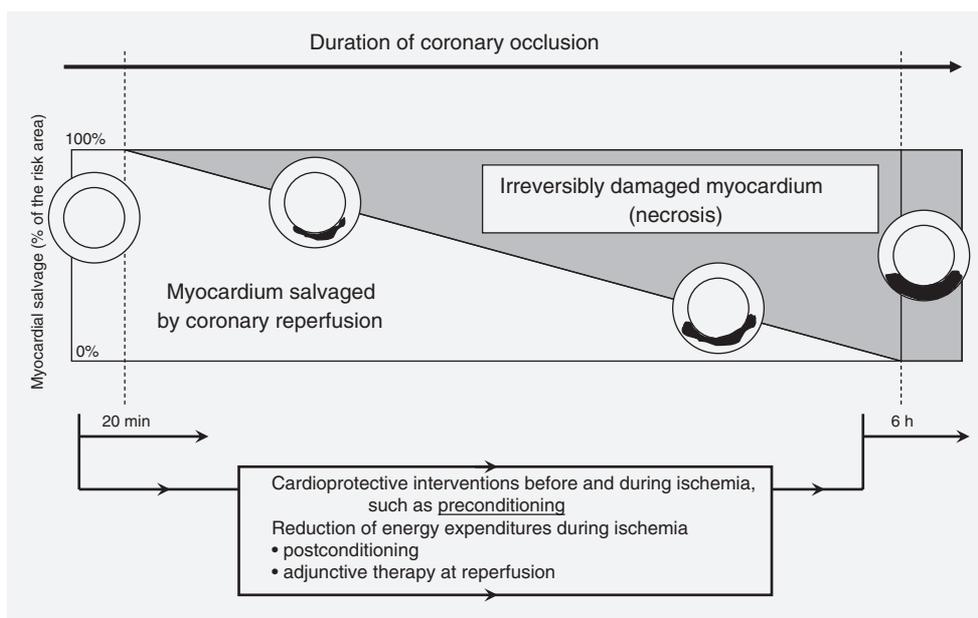


Figure 1. Schematic diagram illustrating the fundamental relationship between myocardial salvage and the time point of initiating coronary artery reperfusion in the dog. Myocardial salvage may be complete after very brief durations of ischemia; however, a progression of myocardial necrosis from the subendocardium towards the subepicardium occurs thereafter. When the coronary artery is reperfused very late, almost the entire area at risk becomes necrotic. Several factors and therapeutic interventions may shift the time frame into the direction of increased tolerance towards the ischemic insult.

species with negligible collateral flow, or when energy demands during ischemia are greater (for example as a result of increased tissue temperature or increased contractility), earlier reperfusion may be required in order to achieve myocardial salvage [7,8]. Moreover, certain cardioprotective interventions before or during ischemia such as ischemic preconditioning or pharmacologic interventions, and hypothermia during ischemia, may extend the period up to which reperfusion still results in complete or partial myocardial salvage [9–11]. In addition, final infarct size may not be invariably determined at the time when reflow is initiated: a “stuttering” reperfusion, termed “postconditioning” and referring to brief re-occlusions of the coronary artery early during the reperfusion period, may result in a smaller infarct size than occurs after an immediate, single and complete re-opening of the occluded artery [12]. The findings of many experimental investigations have also suggested that various adjunctive pharmacologic interventions during the reperfusion period may further reduce the size of the infarct. However, not all laboratories have observed benefits from therapies, such as postconditioning, that are initiated only at reperfusion [13].

Whether initiating reperfusion at a time when myocardial salvage is no longer achievable is of significant benefit (“open artery hypothesis”) remains a matter of debate [14]. Data from experimental studies have suggested that healing of infarcted tissue may be favorably influenced even by late coronary artery reperfusion, which could attenuate infarct expansion,

scar thinning, and aneurysmal dilatation beyond myocardial salvage [15].

In summary, reperfusion of ischemic myocardium will result in necrotic and salvaged myocardium within the risk area, ranging from complete salvage with very early reperfusion to almost complete necrosis with late reperfusion. Necrotic myocytes will eventually be replaced by scar tissue, but salvaged cardiomyocytes are characterized by various abnormalities that will normalize after a variable time course.

## Morphologic and metabolic changes induced by reperfusion

With initiation of reflow, marked hyperemia (up to approximately 500% of basal flow) develops for approximately 15 min, restoring aerobic metabolism in reversibly altered myocardium [2–5]. Re-phosphorylation of adenine nucleotides to adenosine triphosphate, a pronounced increase in creatine phosphate, and normalization of lactate concentrations and pH are accompanied by transient swelling of mitochondria and cardiomyocytes [16,17]. During the first 10 min of reperfusion, oxygen-derived free radicals show a marked peak in concentration [18]. In addition, activation of a pattern of genes occurs during reperfusion, eventually leading to increased tissue concentrations of (for example) heat shock proteins and inducible nitric oxide synthase after 1 day of reperfusion [19].

#### Reversible contractile dysfunction: stunning

Prolonged, postischemic dysfunction of viable myocardial tissue salvaged by reperfusion, termed “stunning”, develops reproducibly after brief periods of ischemia followed by reperfusion. Despite adequate restoration of coronary flow, contractile dysfunction, in particular also diastolic dysfunction, may persist for periods ranging from hours to several days, depending on the duration and completeness of the initial ischemic insult. Besides this typical flow–contraction mismatch in stunning, another of its characteristics is that inotropic stimulation of stunned myocardium results in enhanced contractility without altering further recovery of the myocardium. Experiments using free radical scavengers have indicated that a major part of the stunning effect appears to be the result of desensitizing of the sarcolemmal contractile apparatus caused by the burst in oxygen-derived free radicals – mainly hydroxyl radical – during the first minutes of reperfusion [20]. Therefore, stunning can be described as a form of reperfusion injury that is, at least in part, induced by the reperfusion-induced burst in free radicals. Notably, contractile dysfunction is not completely preventable by oxygen-radical scavenging, which might reflect the incomplete effect of the radical scavengers or might point to additional, yet unidentified mechanisms leading to stunned myocardium.

#### Microvascular alterations

Paradoxically, re-opening of the coronary artery, the prerequisite for restoration of tissue reperfusion, may simultaneously initiate progressive microvascular damage, termed the “no-reflow phenomenon”. Despite complete restoration of epicardial artery patency, discrete perfusion defects may develop within the previously ischemic cardiac tissue during the first hours of reperfusion [21]. In the rabbit, regional myocardial blood flow, which was  $2.06 \pm 0.01$  mL/min per g before ischemia, increased to 3.78 mL/min per g after 2 min of reperfusion following a period of 30 min of coronary occlusion; this was followed by a decline within 2 h of reperfusion, and a final plateau at about 0.9 mL/min per g by 2 and 8 h of reperfusion. Concomitantly, sizeable anatomic perfusion defects developed within the risk area, increasing from 12.2% of the area after 2 min of reperfusion to 30.8% and 34.9% at 2 and 8 h of reperfusion, respectively. These areas of no reflow were contained within the necrotic zone and finally comprised approximately 80% of the infarct size in this model [22]. Several pathophysiologic mechanisms for this phenomenon (including endothelial swelling and bleb formation, progressive leukocyte plugging, and vascular com-

pression by tissue edema) are under debate; however, this progressive vascular obstruction may be regarded as reperfusion injury at the microvascular level, with potentially significant prognostic implications [23].

#### Putting the heart in the “preconditioned” state by reperfusion

Brief coronary occlusions beneath the threshold for irreversible damage, followed by reperfusion, can induce cardioprotective effects via a complex, as yet incompletely understood, cascade of signals that renders the myocardium more tolerant towards a subsequent more prolonged ischemia insult [10]. This process is termed “ischemic preconditioning”. In the dog, infarct size is substantially reduced if an episode of 3–10 min of ischemia, followed by 5 min of reperfusion – the preconditioning procedure – precedes a longer episode of ischemia and reperfusion [24]. The cardioprotection conferred by such a preconditioning stimulus may no longer be detectable if the final ischemic insult is instituted after more than 3 h, but there seems to be a second window of delayed protection, evident if the long-duration coronary occlusion is performed 24–96 h after the preconditioning stimulus [25].

#### Summary

The earlier the coronary reperfusion is established, the greater the portion of myocardium that can be salvaged, thereby limiting the amount of myocardial necrosis. Nonetheless, reperfusion itself may also result in unfavorable consequences. The most important are progressive microvascular alterations (no reflow), and reversible contractile dysfunction of salvaged myocardium (stunning). Reduction of irreversible cardiomyocyte damage may be accomplished by administration of cardioprotective therapies before ischemia, such as preconditioning or drugs that mimic preconditioning or cardioprotective interventions during ischemia, such as hypothermia. Whether modifying the specific procedure for reperfusion (for example, postconditioning) or administering adjunctive therapy only at reperfusion reduces irreversible myocyte injury remains controversial. ■

#### REFERENCES

1. Reimer KA, Jennings RB, Tatum AH. Pathobiology of acute myocardial ischemia: metabolic, functional and ultrastructural studies. *Am J Cardiol.* 1983;52:72A–81A.
2. Kloner RA, Ganote CE, Whalen D, Jennings RB. Effects of a transient period of ischemia on myocardial cells, II: fine structure during the first few minutes of reflow. *Am J Pathol.* 1974;74:399–422.

## Basic article

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- Jennings RB, Schaper J, Hill ML, et al. Effects of reperfusion late in the phase of reversible ischemic injury: changes in cell volume, electrolytes, metabolites, and ultrastructure. *Circ Res*. 1985;56:262–278.
- Reimer KA, Lowe JE, Rasmussen MM, Jennings RB. The wave-front phenomenon of ischemic cell death. 1. Myocardial infarct size vs duration of coronary occlusion in dogs. *Circulation*. 1977;56:786–794.
- Kloner RA, Ellis SG, Lange R, Braunwald E. Studies of experimental artery reperfusion: effects on infarct size, myocardial function, biochemistry, ultrastructure and microvascular damage. *Circulation*. 1983;68:18–115.
- Przyklenk K, Vivaldi MT, Schoen FJ, Malcolm J, Arnold O, Kloner RA. Salvage of ischaemic myocardium by reperfusion: importance of collateral blood flow and myocardial oxygen demand during occlusion. *Cardiovasc Res*. 1986;20:403–414.
- Miura T, Downey JM, Ooiwa H, Adachi T, Noto T, Shizukuda Y. Progression of myocardial infarction in a collateral deficient species. *Jpn Heart J*. 1989;30:695–708.
- Muller KD, Sass S, Gottwik MG, Schaper W. Effect of myocardial oxygen consumption on infarct size in experimental coronary artery occlusion. *Basic Res Cardiol*. 1982;77:170–181.
- Yao Z, Gross GJ. A comparison of adenosine-induced cardioprotection and ischemic preconditioning in dogs. Efficacy, time course, and role of KATP channels. *Circulation*. 1994;89:1229–1236.
- Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation*. 1986;74:1124–1136.
- Hale SL, Kloner RA. Myocardial temperature reduction attenuates necrosis after prolonged ischemia in rabbits. *Cardiovasc Res*. 1998;40:502–507.
- Zhao ZQ, Corvera JS, Halkos ME, et al. Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. *Am J Physiol Heart Circ Physiol*. 2003;285:H579–H588.
- Dow J, Kloner RA. Postconditioning does not reduce myocardial infarct size in an in vivo regional ischemia rodent model. *J Cardiovasc Pharmacol Ther*. 2007;12:153–163.
- Hochman JS, Lamas GA, Buller CE, et al., for the Occluded Artery Trial Investigators. Coronary intervention for persistent occlusion after myocardial infarction. *N Engl J Med*. 2006;355:2395–2407.
- Hochman JS, Choo H. Limitation of myocardial infarct expansion by reperfusion independent of myocardial salvage. *Circulation*. 1987;75:299–306.
- Fleet WF, Johnson TA, Graebner CA, et al. Effects of serial brief ischemic episodes on extracellular K<sup>+</sup>, pH, and activation in the pig. *Circulation*. 1985;72:922–932.
- Allison TB, Ramey CA, Holsinger JW Jr. Transmural gradients of left ventricular tissue metabolites after circumflex artery ligation in dogs. *J Mol Cell Cardiol*. 1977;9:837–852.
- Bolli R, Patel BS, Jeroudi MO, Lai EK, McCay PB. Demonstration of free radical generation in “stunned” myocardium of intact dogs with the use of the spin trap alpha-phenyl N-tertiary butyl nitron. *J Clin Invest*. 1988;82:476–485.
- Marber MS, Latchman DS, Walker JM, Yellon DM. Cardiac stress protein elevation 24h after brief ischemia or heat stress is associated with resistance to myocardial infarction. *Circulation*. 1998;88:1264–1272.
- Bolli R, Jeroudi MO, Patel BS, et al. Marked reduction of free radical generation and contractile dysfunction by antioxidant therapy begun at the time of reperfusion: evidence that myocardial “stunning” is a manifestation of reperfusion injury. *Circ Res*. 1989;65:607–622.
- Ambrosio G, Weisman HF, Mannisi JA, Becker LC. Progressive impairment of regional myocardial perfusion after initial restoration of postischemic blood flow. *Circulation*. 1989;80:1846–1861.
- Reffelmann T, Kloner RA. Microvascular reperfusion injury: rapid expansion of anatomic no reflow during reperfusion in the rabbit. *Am J Physiol Heart Circ Physiol*. 2002;283:H1099–H1107.
- Reffelmann T, Hale SL, Dow JS, Kloner RA. No-reflow phenomenon persists long-term after ischemia/reperfusion in the rat and predicts infarct expansion. *Circulation*. 2003;108:2911–2917.
- Jennings RB, Sebbag L, Schwartz LM, Crago MS, Reimer KA. Metabolism of preconditioned myocardium: effects of loss and reinstatement of cardioprotection. *J Mol Cell Cardiol*. 2001;33:1571–1588.
- Kuzuya T, Hoshida S, Yamashita N, et al. Delayed effects of sublethal ischemia on the acquisition of tolerance to ischemia. *Circ Res*. 1993;72:1293–1299.