

# The paradoxes of reperfusion in the ischemic heart

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## Abstract

Although reperfusion is essential for salvage of the ischemic heart, reperfusion beyond a certain period of ischemia may cause injury to the myocardium. Dramatic changes in contractile function, arrhythmias, and ultrastructure occur in the ischemic-reperfused heart as a consequence of the generation of oxyradicals, loss of cation homeostasis, depletion of energy stores, and changes in subcellular activities. These acute effects of reperfusion appear to be the result of the occurrence of oxidative stress and intracellular  $\text{Ca}^{2+}$  overload in the heart. Alterations in cardiac gene expression may account for delayed recovery of subcellular organelles in the ischemic myocardium.

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## Introduction

Myocardial ischemia occurs when there is a reduction of blood flow as a result of narrowing of the coronary arteries during atherosclerosis, blockage of perfusion as a result of thrombosis, or development of coronary spasm. It is well known that myocardial ischemia results in the loss of contractile function and produces myocardial damage as a consequence of cell death from both necrosis and apoptosis [1–6]. It is therefore essential to restore coronary flow to the ischemic myocardium by interventions such as angioplasty, thrombolytic treatment or coronary bypass surgery [1]. However, if the coronary flow is not restored within a critical period of time, reperfusion itself may cause a wide variety of harmful effects in the ischemic heart – a

phenomenon referred to as “reperfusion injury” [4–6]. For example, reperfusion of ischemic tissue may intensify pathological processes that contribute to the generation of oxyradicals, disturbances in cation homeostasis, and depletion of cellular energy stores, which may elicit arrhythmias, contractile dysfunction, and ultrastructural damage, in addition to endothelial dysfunction and coronary vasoconstriction (*Figure 1*). These paradoxical effects of reperfusion become evident quite rapidly upon the restoration of perfusion [7–11], and may last for periods of several weeks to months. In particular, depressed cardiac function (myocardial stunning) of the ischemic-reperfused heart is commonly seen under chronic conditions after coronary bypass surgery or in patients with a heart transplant.

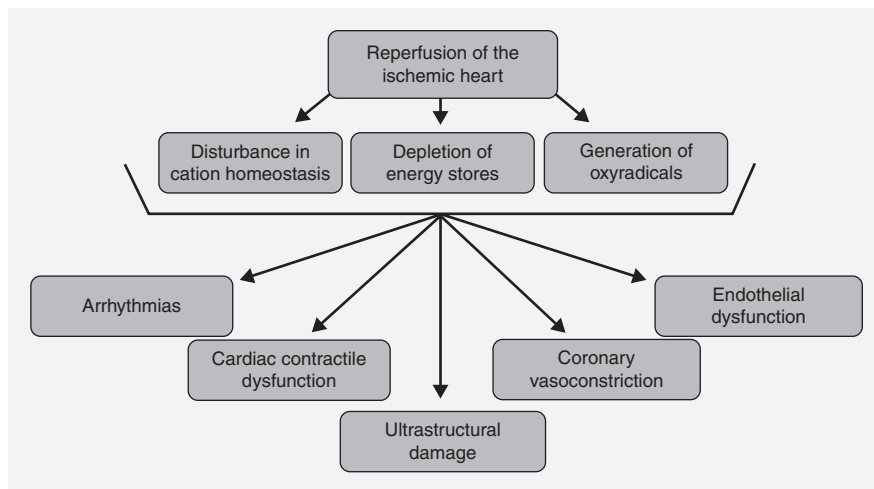


Figure 1. Paradoxical effects of reperfusion in the ischemic heart.

## Ischemic injury or reperfusion injury

As ischemia reduces the delivery of oxygen and substrates to the myocardium, the initial process contributing to ischemic injury is a reduction of cellular energy status as a result of compromised mitochondrial production of ATP via impaired oxidative phosphorylation and electron transport [1,2,9,10]. Although there is an increase in anaerobic glycolysis in ischemic hearts, this metabolic change is not sufficient to meet the high energy demands for maintaining cardiac function [1,5,10]. Derangement of the mitochondrial electron transport system also promotes the generation of oxyradicals and development of oxidative stress in the ischemic heart [6,12]. Nonetheless, increased anaerobic glycolysis is considered to result in the accumulation of  $H^+$ , stimulation of the  $Na^+-H^+$  exchanger, activation of the  $Na^+-Ca^{2+}$  exchanger in the reverse mode, and development of intracellular  $Ca^{2+}$  overload [3,6]. Oxidative stress has also been shown to produce intracellular  $Ca^{2+}$  overload, and vice versa.

The degree of oxidative stress and the magnitude of intracellular  $Ca^{2+}$  overload in cardiomyocytes seem to be dependent upon the duration of ischemia. In fact, both oxidative stress and intracellular  $Ca^{2+}$  overload are considered to be the major mechanisms for the development of ischemic injury, and reperfusion appears to exacerbate the impact of these pathological processes [2,6,7]. Thus it is probable that ischemic injury and reperfusion injury are two facets of the same problem, in which ischemic injury is associated with alterations in myocardial metabolism, including depletion of energy stores, and the reperfusion injury is associated with additional changes, including the development of oxidative stress and the occurrence of intracellular  $Ca^{2+}$  overload (Figure 2).

## Oxidative stress and the ischemia-reperfusion injury

Reactive oxygen species (ROS) are short-lived oxygen-derived free radicals, which act as signaling molecules at low concentrations, but adversely influence cell function by promoting lipid peroxidation and the oxidation of cardiac proteins when present in high concentrations. The major sources of ROS in the ischemic heart include the enzyme xanthine oxidase, the mitochondrial electron transport chain, and the NADPH oxidase system [8]. Oxidative stress may increase by as much as 100-fold during ischemia and reperfusion, as ROS production is greatly accelerated [8,12,13]. In addition, the cellular conditions associated with ischemia-reperfusion favor the

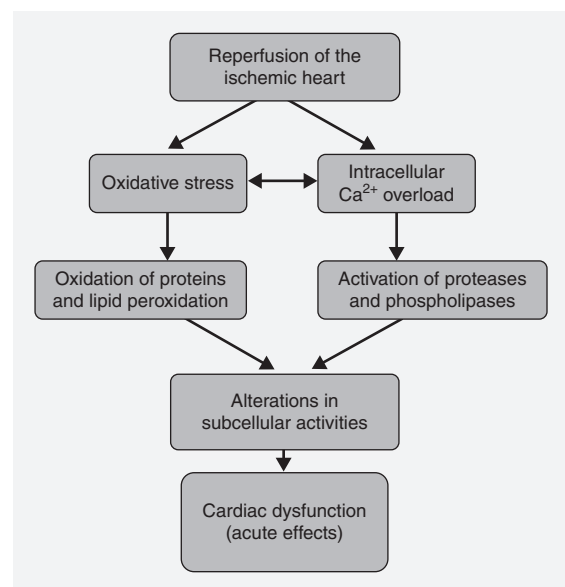


Figure 2. Mechanisms for the acute effects of reperfusion in the ischemic myocardium.

conversion of less reactive oxidants to more reactive species (e.g., the conversion of hydrogen peroxide to hydroxyl radicals or nitric oxide to peroxynitrite) [13,14]. These increases in ROS concentration and potency overwhelm the intracellular free-radical scavenger systems and leads to cellular injury during ischemia-reperfusion.

It has been demonstrated that ROS contribute to altered cation homeostasis through direct effects on membrane proteins involved in the regulation of cation transport and lipid peroxidation, which causes a change in membrane permeability [5] in addition to an increased inflammatory response to ischemia-reperfusion as a result of leukocyte activation. Prolonged oxidative stress also exerts chronic effects, as ischemia-reperfusion has been shown to produce expression of cardiac genes and associated remodeling of subcellular organelles [15,16] (Figure 3). Therapeutic treatments such as the administration of antioxidants or the overexpression of proteins that attenuate oxidative stress have been found to enhance the recovery of contractile function and to reduce infarct size, during ischemia and reperfusion [6,17].

### Calcium overload and ischemia-reperfusion injury

Accumulation of intracellular  $\text{Na}^+$  and  $\text{Ca}^{2+}$  during ischemia occurs in response to the loss of energy homeostasis and as a result of changes in the acute regulation of sarcolemmal and sarcoplasmic reticulum cation transport mechanisms. Specifically, ischemia reduces the activity of sarcolemmal  $\text{Na}^+/\text{K}^+$ -ATPase,

increases the activity of the  $\text{Na}^+-\text{H}^+$  exchanger, and promotes the activation of the  $\text{Na}^+-\text{Ca}^{2+}$  exchanger in a reverse mode. Calcium transport is also affected by ischemia-reperfusion, because oxidative stress adversely influences  $\text{Ca}^{2+}$ -handling proteins in the sarcoplasmic reticulum ( $\text{Ca}^{2+}$  pump, sarcoplasmic reticulum  $\text{Ca}^{2+}$ -ATPase, and the  $\text{Ca}^{2+}$  release channel) and sarcolemma (sarcolemmal  $\text{Ca}^{2+}$  pump and the L-type  $\text{Ca}^{2+}$  channels) [6,18] and thus contributes to the development of an intracellular  $\text{Ca}^{2+}$  overload.

The pathological effects induced by intracellular  $\text{Ca}^{2+}$  overload are mediated by  $\text{Ca}^{2+}$ -induced activation of membrane phospholipases and proteases, and these changes may explain the acute effects of ischemia-reperfusion injury (Figure 2). Mitochondrial dysfunction [19] also contributes to the pathological effects associated with ischemia, because it is known that intracellular  $\text{Ca}^{2+}$  overload influences the opening of mitochondrial  $\text{K}_{\text{ATP}}$  channels and mitochondrial permeability transition pores, which activate apoptotic pathways [20]. Therapeutic treatments such as  $\text{Ca}^{2+}$  antagonists [21] and  $\text{Na}^+-\text{H}^+$  exchange inhibitors [22], which attenuate intracellular  $\text{Ca}^{2+}$  overload, have been suggested to reduce the size of infarcts resulting from ischemia-reperfusion. Thus the development of intracellular  $\text{Ca}^{2+}$  overload can be seen to contribute to the acute effects of ischemia-reperfusion injury.

### Subcellular remodeling in the ischemic-reperfused heart

During prolonged ischemia-reperfusion, significant changes in expression of cardiac genes occur such that subcellular organelles (eg, mitochondria, myofibrils, sarcolemma, and sarcoplasmic reticulum) are remodeled (Figure 3). As both oxidative stress and intracellular  $\text{Ca}^{2+}$  overload induced by ischemia-reperfusion are known to produce changes in gene expression, these factors seem to contribute to the remodeling of subcellular organelles and lead to a delayed recovery of contractile function [1,2,6]. These effects are not limited to the cardiomyocyte, as the pathological process may be amplified by the activation of neutrophils, which promote the formation of proinflammatory mediators, oxygen radicals, and the reduction of nitric oxide formation in the endothelium, leading to coronary constriction. Reperfusion-induced functional changes in vascular smooth muscle may also compound the injury process, as altered vascular reactivity may adversely influence the coronary flow and may result in a "no reflow" phenomenon. It may be noted that myocardial stunning, myocardial infarction [9,10,23], and potentially lethal arrhythmias are also associated with reperfusion of the ischemic heart [11]. Although preconditioning,

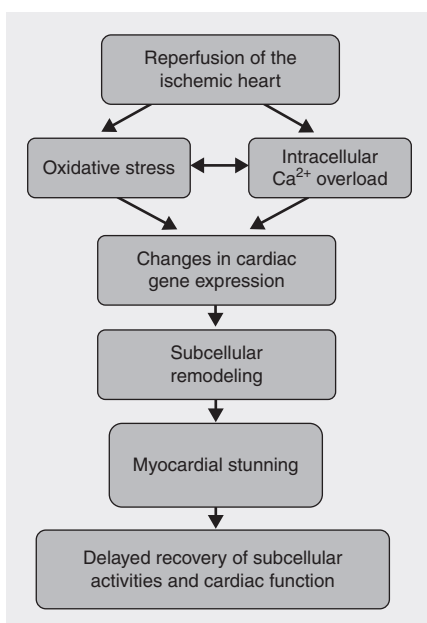


Figure 3. Mechanisms for the chronic effects of reperfusion (myocardial stunning) in the ischemic myocardium.

which is achieved by subjecting the heart to brief periods of ischemia, is known to limit both the size of the infarct and the cardiac dysfunction caused by ischemia-reperfusion [24], it is difficult to use this experimental intervention in clinical settings. Because  $\text{Ca}^{2+}$  handling by cardiomyocytes from ischemic reperfused heart becomes defective as a consequence of oxidative stress [25], it is to be emphasized that both intracellular  $\text{Ca}^{2+}$  overload and oxidative stress should be targeted, to avoid the adverse effects of ischemia-reperfusion injury.

### Summary

Reperfusion of ischemic myocardium is invariably associated with arrhythmias and cardiac dysfunction as a consequence of the generation of oxyradicals, disturbance in cation homeostasis, and depletion of energy stores. Marked alterations in expression of cardiac genes as a result of ischemia-reperfusion are seen to explain subcellular remodeling and delayed recovery of cardiac function. These paradoxical effects of reperfusion appear to be attributable to the occurrence of oxidative stress and intracellular  $\text{Ca}^{2+}$  overload in the myocardium. ■

### REFERENCES

1. Jennings RB, Reimer KA. The cell biology of acute myocardial ischemia. *Annu Rev Med.* 1991;42:225–246.
2. Hearse DJ, Bolli R. Reperfusion induced injury: manifestations, mechanisms, and clinical relevance. *Cardiovasc Res.* 1992;26:101–108.
3. Dhalla NS, Elmoselhi AB, Hata T, Makino N. Status of myocardial antioxidants in ischemia-reperfusion injury. *Cardiovasc Res.* 2000;47:446–456.
4. Piper HM, Meuter K, Schafer C. Cellular mechanisms of ischemia-reperfusion injury. *Ann Thorac Surg.* 2003;75: S644–S648.
5. Buja LM. Myocardial ischemia and reperfusion injury. *Cardiovasc Pathol.* 2005;14:170–175.
6. Dhalla NS, Saini HK, Tappia PS, Sethi R, Mengi SA, Gupta SK. Potential role and mechanisms of subcellular remodeling in cardiac dysfunction due to ischemic heart disease. *J Cardiovasc Med.* 2007;8:238–250.
7. Hoffman JW Jr, Gilbert TB, Poston RS, Silldorff EP. Myocardial reperfusion injury: etiology, mechanisms, and therapies. *J Extra Corpor Technol.* 2004;36:391–411.
8. Zweier JL, Talukder MA. The role of oxidants and free radicals in reperfusion injury. *Cardiovasc Res.* 2006;70:181–190.
9. Hansen PR. Myocardial reperfusion injury: experimental evidence and clinical relevance. *Eur Heart J.* 1995;16:734–740.
10. Ferrari R. Metabolic disturbances during myocardial ischemia and reperfusion. *Am J Cardiol.* 1995;76:17B–24B.
11. Manning AS, Hearse DJ. Reperfusion-induced arrhythmias: mechanisms and prevention. *J Mol Cell Cardiol.* 1984;16:497–518.
12. Zweier JL, Flaherty JT, Weisfeldt ML. Direct measurement of free radical generation following reperfusion of ischemic myocardium. *Proc Natl Acad Sci U S A.* 1987;84:1404–1407.
13. Kuppasamy P, Zweier JL. Characterization of free radical generation by xanthine oxidase. Evidence for hydroxyl radical generation. *J Biol Chem.* 1989;64:9880–9884.
14. Wang P, Zweier JL. Measurement of nitric oxide and peroxynitrite generation in the postischemic heart. Evidence for peroxynitrite-mediated reperfusion injury. *J Biol Chem.* 1996;271: 29223–29230.
15. Temsah RM, Netticadan T, Chapman D, Takeda S, Mochizuki S, Dhalla NS. Alterations in sarcoplasmic reticulum function and gene expression in ischemic-reperfused rat heart. *Am J Physiol Heart Circ Physiol.* 1999;277:H584–H594.
16. Elmoselhi AB, Lukas A, Ostadal P, Dhalla NS. Preconditioning attenuates ischemia-reperfusion induced remodeling of  $\text{Na}^+/\text{K}^+$ -ATPase in hearts. *Am J Physiol Heart Circ Physiol.* 2003;286:H1055–H1063.
17. Ambrosio G, Weisfeldt ML, Jacobus WE, Flaherty JT. Evidence for a reversible oxygen radical-mediated component of reperfusion injury: reduction by recombinant human superoxide dismutase administered at the time of reflow. *Circulation.* 1987;75:282–291.
18. Bersohn MM, Morey AK, Weiss RS. Sarcolemmal calcium transporters in myocardial ischemia. *J Mol Cell Cardiol.* 1997;29:2525–2532.
19. Makazan Z, Saini HK, Dhalla NS. Role of oxidative stress in alterations in mitochondrial function in the ischemic reperfused hearts. *Am J Physiol Heart Circ Physiol.* 2007;292:H1986–H1994.
20. Minezaki KK, Suleiman MS, Chapman RA. Changes in mitochondrial function induced in isolated guinea-pig ventricular myocytes by calcium overload. *J Physiol.* 1994;476:459–471.
21. Urquhart J, Epstein SE, Patterson RE. Comparative effects of calcium-channel blocking agents on left ventricular function during acute ischemia in dogs with and without congestive heart failure. *Am J Cardiol.* 1985;55:10B–16B.
22. Avkiran M, Snabaitis AK. Regulation of cardiac sarcolemmal  $\text{Na}^+/\text{H}^+$  exchanger activity: potential pathophysiological significance of endogenous mediators and oxidant stress. *J Thromb Thrombolysis.* 1999;8:25–32.
23. Bolli R, Marban E. Molecular and cellular mechanisms of myocardial stunning. *Physiol Rev.* 1999;79:609–634.
24. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation.* 1986;74:1124–1136.
25. Saini HK, Dhalla NS. Defective calcium handling in cardiomyocytes isolated from hearts subjected to ischemia-reperfusion. *Am J Physiol Heart Circ Physiol.* 2005;288: H2260–H2270.