Reperfusion Injury: Basic Concepts and Protection Strategies

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Abstract. Prolonged ischemia such as that following myocardial infarction or occurring during long-term coronary bypass procedures causes serious damage to the myocardium. Early reperfusion is an absolute prerequisite for the survival of ischemic tissue. However, reperfusion has been referred to as the "double edged sword" because reperfusing ischemic myocardium carries with it a component of injury known as reperfusion injury. Reperfusion injury includes a number of events, such as reperfusion arrhythmias, myocardial infarction, stunning, vascular damage, and endothelial dysfunction. The underlying mechanism of reperfusion injury is not entirely known, but the existing evidence suggests that oxygen free radicals generated during the first few minutes of reflow lead to damage of cellular membranes, intracellular calcium overload, and uncoupling of excitation-contraction coupling. Although controversial, free radical scavengers, in general, are highly effective in the attenuation of reperfusion injury in animal models. Newer endogenous protection strategies, which include ischemic and heat shock preconditioning, are known to reduce reperfusion injury following ischemia.

Key Words. ischemia, reperfusion injury, calcium, lipid peroxidation, antioxidants

A major concern during cardiopulmonary bypass, coronary reperfusion with thrombolytic therapy, and percutaneous transluminal angioplasty procedures is minimizing ischemic damage to the myocardium, thereby avoiding depressed myocardial performance in the reperfusion period. Prolonged ischemia, such as that following myocardial infarction or occurring during long-term coronary bypass procedures, can cause serious damage to the myocardium. Reperfusion is an absolute requirement for the survival of the ischemic myocardium. Unfortunately, reperfusion has also been referred to as a "double edged sword" [1] because abundant evidence suggests that reperfusing ischemic myocardium carries with it a component of injury known as reperfusion injury.

Concerns about myocardial reperfusion were expressed by Jennings et al. [2] in 1960 when they suggested that reperfusion may accelerate the development of necrosis in irreversibly injured myocytes. They observed an ultrastructural appearance of "explosive swelling," which included architectural disruption, contraction bands, and intramitochondrial calciunm phosphate granules. In 1977, Bulkley and Hutchins [3] reported the paradox is of myocardial necrosis after successful revascularization by coronary artery bypass graft surgery and suggested that the lesions were surgery related and represented contact due to calcium loading and myocardial cellular edema in the distribution of widely patent arterial grafts. They further concluded that "prevention of intraoperative myocardial injury must also focus on characteristics of the phase of myocardial reperfusion." Greene and Weisfeldt [4] and Shine and Douglas [5] demonstrated that measures instituted after the termination of ischemia that attenuated the rise in myocardial cytosolic calcium led to a reduction of tissue injury. Subsequently other workers have implicated cell swelling, white blood cell plugging of vessel, endothelial cell damage, and free radical damage in reperfusion injury of the heart.

Mediators of Reperfusion Injury

Two popular theories of how reperfusion injury might occur are the calcium hypothesis and the free radical hypothesis [6]. The former suggests that ischemia induces a defect in the cell's ability to regulate calcium such that upon reperfusion the cell accumulates toxic levels of calcium. The free radical hypothesis is based on the premise that partially reduced forms of molecular oxygen, including the superoxide anion (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), and hydroxyl radical (OH), are produced at the time of reperfusion. Many investigators, but not all [7], have found that free radical scavengers such as superoxide dismutase (SOD), with or without catalase, can reduce the injury in the isolated heart model and argue in favor of a free radical mechanism. In fact, free radicals might be inducing the membrane defects that promote calcium entry, thus unifying both these theories.

Free radicals

Free radicals, atoms or molecules with unpaired electrons that are capable of independent existence, can
be formed through either loss or acquisition of a single electron. The presence of one or more unpaired electrons makes these compounds highly reactive. In the case of oxygen, its univalent reduction leads to the formation of superoxide anion, serving a key role in the generation of other more reactive species. It is thought to be cytotoxic and is a requirement for the production of the -OH radical. The hydroxyl radical can be formed by the Haber-Weiss reaction when an \( \cdot O_2^- \) anion and an \( H_2O_2 \) molecule spontaneously combine to form molecular oxygen and two \( \cdot OH \) radicals. A much more efficient method of producing the \( \cdot OH \) radical is via the Fenton reaction, where \( H_2O_2 \) accepts an electron from a reduced metal ion such as \( Fe^{2+} \). Superoxide serves a critical role here as well because it is the primary reducing agent for replenishing the reduced metal ion. Recently, a second pathway [8] for the generation of an \( \cdot OH \) radical has been described, in which \( \cdot OH \) is generated from an iron-independent reaction involving the interaction of \( \cdot O_2^- \) and nitric oxide (NO), the latter being a similar reaction to the endothelial cell–derived relaxing factor. The proposed chemistry is as follows:

\[
\begin{align*}
NO + \cdot O_2^- & \rightarrow ONOO^- \text{ (peroxynitrite anion)} \\
ONOO^- + H & \rightarrow ONOOH \text{ (peroxynitrosic acid)} \\
ONOOH & \rightarrow \cdot OH + NO_2 \text{ (nitrogen dioxide)}
\end{align*}
\]

This reaction process ultimately produces nitrates and nitrates. The high reactivity of the \( \cdot OH \) radical causes it to react at diffusion-limited rates, and it reacts with the first molecule it comes into contact (usually within 14 A following a period of \( 10^{-6} \) second). In acid media, such as the vacuole of the phagocyte or the microenvironment of cell membranes (the cell surface, containing polyanions, attracts \( H^+ \) ions), \( \cdot O_2^- \) is protonated into the perhydroxy radical \( HOO_2^- \). The \( HOO_2^- \) radical is a stronger oxidant than \( \cdot O_2^- \), and is cytotoxic. Singlet oxygen \( (1^O_2) \) is formed if one of the unpaired electrons of molecular oxygen is transferred via energy absorption to higher energy orbitals and its spin is inverted. Singlet oxygen exists in two states, that is, as delta \( (\Delta) \) \( ^1O_2 \), in which the newly paired electrons occupy the same orbital, and as sigma \( (\Sigma) \) \( ^1O_2 \), in which the electrons of opposite spins are in different orbitals. The lifetime of \( ^1O_2 \) is sufficiently short that all \( ^1O_2 \) chemistry in solution involves the \( ^1O_2 \) state. The surplus energy of \( ^1O_2 \) may be spent through thermal decomposition, light emission, or chemical reaction (as indicated by chemiluminescence) [9,10]. The importance of various pathways of free radical production in this condition and the hierarchical roles of different active oxygen intermediates in the damage are currently in dispute.

Several mechanisms have been described for the production of free radicals in biological tissues. Some of the widely accepted mechanisms are as follows:

- **xanthine oxidase** [11–13], activated neutrophils [14],
- **direct donation of electrons from the reduced mitochondrial electron transport chain (NADH dehydrogenase, ubiquinone-cytochrome b regions) to molecular oxygen** [15], catecholamine oxidation [16], cytochrome, and lipoxygenase enzymes (prostaglandins) [17]. The importance of various pathways of free radical production during ischemia/reperfusion and the hierarchical roles of different active oxygen intermediates in the damage are currently in dispute. Also, it is apparent from various studies that the ultimate source(s)/mechanism(s) of free radical production is (are) highly dependent on the injury model used. For example, it is unlikely that free radicals produced by the isolated perfused post-ischemic heart originate from leukocytes [18], and in the neonatal myocyte, free radical production is from endothelial cells [19]. All of these sources are able to generate \( \cdot O_2^- , H_2O_2 , \cdot OH , \) and NO radical. The highly reactive \( \cdot OH \) radical and \( O_2^- \) initiate lipid peroxidation (LOOH) of cell membrane components, causing the release of proinflammatory mediators that activate, attract, and promote the adherence of neutrophils to the vascular endothelium [20]. The neutrophils release oxidants and elastase, which further damage myocytes (Figure 1).

### How do free radicals induce myocardial injury?

Alterations in membrane proteins by free radicals are among the important factors in the evolution of myocardial ischemia/reperfusion damage. Membranes are composed mostly of phospholipids and proteins. Oxygen free radicals can attack subcellular structures, resulting in metabolic and structural changes, leading ultimately to cell death and necrosis (see Figure 3). One well-documented process by which oxygen radicals cause cell injury is microsomal lipid peroxidation, with “oxidative deterioration of polysaturated lipids” [21]. Cell membranes contain large amounts of polysaturated fatty acid complexed to phospholipid, which when peroxidized result in loss of cell integrity and function. Mechanistically, \( \cdot OH \) attacks unsaturated fatty acids, abstracting an hydrogen atom to generate a carbon-centered radical (equation 4).

\[
\text{Lipid-H} + \cdot \text{OH} \rightarrow \text{H}_2\text{O} + \text{lipid-H}
\]

Molecular rearrangement results in the formation of a conjugated diene, with uptake of an \( O_2 \) at its center, to yield an oxygen-centered lipid peroxyl radical (lipid-OO-) (equation 5):

\[
\text{Lipid}^\cdot + O_2 \rightarrow \text{lipid-OO}^\cdot
\]

This peroxyl radical (lipid-OO-) is capable of propagating a chain reaction by extracting a hydrogen atom from another fatty acid molecule (equation 6).

\[
\text{Lipid-OO}^\cdot + \text{lipid-H} \rightarrow \text{lipid-OOH} + \text{lipid-H}
\]