Postconditioning
A new link in nature’s armor against myocardial ischemia-reperfusion injury

Abstract  Reperfusion injury is a complex process involving several cell types (endothelial cells, neutrophils, and cardiomyocytes), soluble pro-inflammatory mediators, oxidants, ionic and metabolic dyshomeostasis, and cellular and molecular signals. These participants in the pathobiology of reperfusion injury are not mutually exclusive. Some of these events take place during the very early moments of reperfusion, while others, seemingly triggered in part by the early events, are activated within a later timeframe. Postconditioning is a series of brief mechanical interruptions of reperfusion following a specific prescribed algorithm applied at the very onset of reperfusion. This algorithm lasts only from 1 to 3 minutes depending on species. Although associated with re-occlusion of the coronary artery or re-imposition of hypoxia in cell culture, the reference to ischemia has been dropped. Postconditioning has been observed to reduce infarct size and apoptosis as the “end games” in myocardial therapeutics; salvage of infarct size was similar to that achieved by the gold standard of protection, ischemic preconditioning. The cardioprotection was also associated with a reduction in: endothelial cell activation and dysfunction, tissue superoxide anion generation, neutrophil activation and accumulation in reperfused myocardium, microvascular injury, tissue edema, intracellular and mitochondrial calcium accumulation. Postconditioning sets in motion triggers and signals that are functionally related to reduced cell death. Adenosine has been implicated in the cardioprotection of postconditioning, as has e-NOS, nitric oxide and guanylyl cyclase, opening of K<sub>ATP</sub> channels and closing of the mitochondrial permeability transition pore. Cardioprotection by postconditioning has also been associated with the activation of intracellular survival pathways such as ERK1/2 and PI3 kinase – Akt pathways. Other pathways have yet to be identified. Although many of the pathways involved in postconditioning have also been identified in ischemic preconditioning, some may not be involved in preconditioning (ERK1/2). The timing of action of these pathways and other mediators of protection in postconditioning differs from that of preconditioning. In contrast to preconditioning, which requires a foreknowledge of the ischemic event, postconditioning can be applied at the onset of reperfusion at the point of clinical service, i.e. angioplasty, cardiac surgery, transplantation.

Key words  Postconditioning – preconditioning – reperfusion injury – infarct size
Introduction

**Historical perspective on reperfusion injury and reperfusion therapy**

Reperfusion remains the definitive treatment to attenuate myocardial infarction, contractile dysfunction and apoptosis following ischemia-reperfusion. However, numerous studies have shown that reperfusion itself can initiate both transient and lethal injury following ischemia, i.e. reperfusion injury. Reperfusion injury has been a strategic target of therapy for many years in cardiac surgery. The concept of reperfusion injury was popularized by Buckberg in the late 1970s and early 1980s [23] and thereafter by Braunwald and Kloner [9]. Although myocardial reperfusion injury was avidly investigated by some laboratories, the very existence of reperfusion injury was robustly debated [72]. This lack of scientific consensus conspired with the necessary lag time in adopting translational therapeutics to delay application of reperfusion injury therapeutics for many years. Attention was focused more on ischemia as both a cause of injury and the most relevant window of opportunity to reduce post-ischemic injury. The important revelation that preconditioning the heart with short periods of ischemia could adapt the heart to better tolerate ischemic injury ignited excitement in the field of myocardial protection, but further fixed the scientific gaze on the ischemic interval since the biochemical alterations and signaling pathways seemed to be active at this time.

In 1986, a volume in the *Journal of Thoracic and Cardiovascular Surgery* entitled “Studies of Controlled Reperfusion After Ischemia“ [10] was dedicated to the investigation and application of various strategies to attenuate reperfusion injury in the setting of global and regional ischemia-reperfusion. The concept of attenuating ischemia-reperfusion injury by controlling the conditions (flow rate, temperature) and composition (pH buffers, metabolic substrates, calcium-reducing agents, anti-oxidants) of the (re)perfusion, introduced by Buckberg and many others as part of the surgical myocardial protection armamentarium, forms the basic platform upon which current myocardial protection strategies rest [89]. The overall concept of modified reperfusion incorporates all therapeutics that intervene in one or more of the complex mechanisms involved in reperfusion injury, whether those mechanisms are mechanical or pharmacological in nature. Strategies such as regional hypothermia, alkalotic perfusates, hypocalcemia, metabolic adjuncts (i.e. pyruvate, glucose-insulin, glutamate and aspartate), regional cardioplegia [90] and gradual or “gentle” reperfusion [63] have their roots in this approach.

It has been some 20 years since these strategies were introduced, and still no strategy to modify reperfusion injury has gained clinical acceptance. The failure to translate experimental therapies to the bedside have placed the field of myocardial protection in a precarious position regarding its own survival [8]. Although cardiologists designed methods by which to achieve early reperfusion by thrombolysis and primary angioplasty, the question of whether reperfusion injury offsets some of the benefits of reperfusion was inevitably raised. Reperfusion injury and its treatment are now topics of very intense investigation, stimulated principally by the seminal observation that events occurring within minutes of the onset of coronary artery reperfusion determine, in part, the pathogenesis of vascular endothelial dysfunction [52], contractile dysfunction [86, 87], necrosis [51, 70, 82, 83, 86, 87], and apoptosis [97, 102]. The early moments of reperfusion, therefore, present a window of opportunity for therapeutics targeting these rapidly developing events (reviewed below) [51, 70].

**The early moments of reperfusion: a time of healing or potential injury?**

Reperfusion is necessary for salvage of myocardium from infarction. However, reperfusion itself is associated with numerous events that extend infarct size and other manifestations of injury beyond that observed during equivalent periods of ischemia alone. These events are collectively termed “reperfusion injury”. Many of these events take place in the very early moments of reperfusion, and have been linked directly to cell injury and death. In addition, some events trigger a cascade of events that occur later in the time course of reperfusion injury that ultimately contribute to cell injury and irreversible cell death. Some of these early events are discussed below, and form the major targets for the cardioprotective strategies applied at the onset of reperfusion.

1. **Rapid generation of reactive oxygen species (ROS):** ROS generation has been observed within the first minutes [45] and even seconds [3] of reperfusion. Sources of ROS include activated neutrophils, stressed cardiomyocytes, activated vascular endothelium and to a minor extent perivascular tissue. Neutrophils generate ROS principally through the NADPH oxidase system. Vascular endothelial cells have several enzyme systems that generate ROS, such as the endothelial NAD(P)H oxidase system, xanthine oxidase and eNOS under some conditions.

2. **Osmotic gradient and cell swelling:** An increased transsarcolemmal osmotic gradient is created by the accumulation of metabolic products of anaerobic metabolism [41]. This event occurs concomitantly with other causes of intracellular water and cell volume dysregulation [77], and with water accumulation.