Editorial

Modifying the first minute of reperfusion: potential for myocardial salvage

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See article by Kin et al. [1] (pages 74–85) in this issue.

In this issue of Cardiovascular Research, Kin et al. [1] have demonstrated in vivo in rats that following a 30-min index regional myocardial ischemia, protection against reperfusion injury assessed 3 h later through necrosis quantification by tetrazolium staining was achieved by a 1-min duration reperfusion modification protocol that consisted of three cycles of 10 s each of unrestricted reperfusion followed by 10 s of re-occlusion of the same coronary artery subjected to the occlusion causing the index ischemia. This protection, while evident, was substantially less than that achieved by one cycle of preconditioning (5 min of ischemia followed by 10 min of reperfusion immediately preceding the index ischemia). Importantly, delaying the three-cycle (10-s ‘on’/10-s ‘off’) reperfusion modification protocol by 1 min (its duration) failed to protect the myocardium at risk against infarction. Furthermore, doubling the reperfusion modification protocol to six cycles (10-s ‘on’/10-s ‘off’) produced no measurable improvement in protection compared to three cycles. These findings focusing on events in the first minute of reperfusion are, in our view, the novel and potentially important contribution of this paper.

The same group, led by Dr. Vinten-Johansen, has previously reported in the dog a substantial reduction in infarct size as a percentage of region at risk, producing about the same amount of protection as single-cycle ischemic preconditioning (same duration as in the rats [1]), with a three-cycle, 30-s unrestricted reperfusion/30-s re-occlusion protocol immediately upon reperfusion after a 60-min index regional ischemia [2]. However, when this reperfusion modification protocol was delayed by 5 min, the protection was lost. The present paper by Kin et al. [1], as described above, refines the temporal focus to the first minute of reperfusion.

The Vinten-Johansen group has called the reperfusion modification protocols described above “ischemic postconditioning”. This is an appealing phrase; we immediately liked it. However, on further reflection, it is problematic. Preconditioning, whether ischemic or pharmacological, involves a stimulus, then a response (which, while very rapid for classical preconditioning, does take a few minutes), and “memory” (that is, persistence of the new preconditioned state conferring benefits) for many minutes to even a few hours, depending on the species. These particular reperfusion protocols highlight the first minute, or 3 min, of reperfusion, but are likely operating so differently than preconditioning that the “postconditioning” moniker is, arguably, unhelpful. Our preference is to consider these protocols as merely a tighter temporal focus on a long tradition of early reperfusion modification efforts.

Whatever language is used to describe this early reperfusion intervention, the most important point is to understand how it works. We agree with these authors that the efficacy of this mechanical reperfusion modification most likely relates to the burst of free radical generation very early during reperfusion. Although the ultimate extent of cardiomyocyte cell death that occurs following ischemia/reperfusion is no doubt multifactorial and certainly not exclusively determined in the first minute, or even 3 min, of reperfusion (an issue to which we shall return), it seems reasonable that the fate of numerous cardiomyocytes still reversibly injured at the onset of reperfusion could be decided by the amount of membrane injury from reactive oxygen species (ROS) initiated in the first minute of reperfusion. Zweier’s pioneering spin resonance measurements of the free radical pulse on reperfusion in hearts [3], which demonstrated a peak of superoxide formation at about 10 to 20 s of reperfusion, are consistent with the importance of the first minute of reperfusion. While the Vinten-Johansen group draws attention to free radical production [1,2] and cite Zweier, they do not focus in their discussion on the
interplay between the generation of ROS versus endogenous scavenging mechanisms (e.g. catalase, superoxide dismutase, glutathione) to effectively neutralize ROS to prevent (or at least delay) irreversible cell injury. We suspect that the brief episodes (10 or 30 s) of re-occlusion are making the critical difference for some cells in preventing the ROS-scavenging mechanisms from being overwhelmed; those 10- or 30-s “time-outs” may be both blunting the burst amplitude of ROS and allowing time for scavenging “catch up”. The measurements of plasma malondialdehyde (marking lipid peroxidation) and dihydroethidium fluorescence myocardial staining (marking superoxide anion production) by the authors [1] after 3-h reperfusion cannot address the dynamics of very early reperfusion, as it is well established that substantial ROS production occurs throughout at least the first few hours of reperfusion [4]. The authors’ measurement of myocardial myeloperoxidase activity (marking neutrophil accumulation) is also not revealing. Evidence is lacking for a causal role of neutrophils in myocardial ischemia/reperfusion injury [5]. There have been both positive and negative reports on free radical scavengers in reducing myocardial injury [6], which might be interpreted as raising some doubts about the importance of ROS. Our view, given the redundancy in sources of free radicals, issues surrounding intracellular delivery of scavengers, and the timing of effective delivery regarding very early reperfusion, is that the early ROS burst upon reperfusion remains a plausible source of significant cardiomyocyte cell death determined within the first minute of reperfusion.

Much more is occurring during reperfusion than membrane injury from ROS. For one thing, there is a sudden increase in the trans-sarcolemmal osmotic gradient between the intracellular and extracellular milieu created by the return of iso-osmotic blood at a time when the intracellular osmolarity of cardiomyocytes has increased during ischemia due to the accumulation of metabolic end products [7]. The explosive swelling that has been observed in cardiomyocytes upon reperfusion following prolonged ischemia appears to play a critical role in a substantial amount of cardiomyocyte death occurring in the first few minutes of reperfusion (e.g., 2 min after 40 min of regional ischemia in the dog [8]), as many cardiomyocytes, already damaged by ischemia, may not be able to withstand the further osmotic swelling upon reperfusion, leading to cell membrane rupture and death. This process may be accentuated by additional injury from ROS during early reperfusion.

Recent observations of changes in cultured cardiomyocyte volume during simulated ischemia in our laboratory consistently show substantial reduction in ischemia-induced cell swelling (no swelling on average in the first 30 min) by ischemic preconditioning as determined from cell volume measurements by laser scanning confocal microscopy. We have demonstrated that this reduction in ischemic cardiomyocyte swelling in vitro is sufficient in magnitude to account for the protection of preconditioning and that the same molecular mechanism mediates both preconditioning and cardiomyocyte regulatory volume decrease (RVD) [9], with chloride channel activity accounting for most of the RVD [10]. We hypothesize that the stimulus of ischemic preconditioning produces the rapid activation of chloride channels in the sarcolemma (resulting in a net flux of Cl⁻ ions, together with osmotically obligated water) immediately from the onset of the index ischemia to explain the above observations of reduced ischemic cell swelling. To maintain electroneutrality, this Cl⁻ efflux must be balanced by a net efflux of positively charged ions, for which K⁺ is the most obvious source. Our studies showing that either chloride channel blockade [11] or inhibition of the inward rectifier current (I_K1) [12] abolish preconditioning protection further support this hypothesis.

We suggest that the transition between reversible and irreversible cell membrane injury, the defining event in oncotic cell death (oncosis), may result from a combination of physical membrane stretching and chemical membrane injury, the latter in part due to ROS generated during early reperfusion. Preconditioned cardiomyocytes, with little swelling at the onset of reperfusion, may have a considerable advantage over cardiomyocytes already swollen almost to the point of rupture as both the ROS burst and the further osmotic stress of reperfusion commence.

Several other mechanisms are important in myocardial muscle cell death during reperfusion. One is contracture, especially calcium-induced contracture, reviewed recently by Piper [13,14], who points out that reduced intracellular acidosis upon reperfusion is important in promoting calcium overload. In this regard, we note that Ambrosio et al. [3] found minimal increase in intracellular pH during the first minute of reperfusion. Another is complement-induced injury [15]. Further, the no-reflow phenomenon [16], whether from ROS-induced injury to endothelium combined with oncotic swelling of these cells, plugging of the microvasculature by erythrocytes and leukocytes, or blood vessel compression by muscle contracture, is important and interesting in that, by these mechanisms, reperfusion may trigger a persistent ischemia that guarantees cell death. We do not think that any of these types of injury are well developed in the first minute of reperfusion. But this is not the point. The real issue is whether the Vinten-Johansen group’s altered reperfusion protocols avoid mechanisms being set in motion that produce cell death later, perhaps after a delay of several minutes or longer, so that cell death is irreversibly determined during the first minute of reperfusion. It is sobering to consider how much myocardium might become unsalvageable after only 1 min of reperfusion when one is considering practical reperfusion modification strategies clinically.

One aspect of the Kin et al. [1] paper is that this group achieved much less myocardial salvage in the rat than in the dog [2] with their reperfusion modification protocols. They suggest that larger amounts of xanthine oxidase in the rat hearts versus dog hearts might explain the difference based on the role of xanthine oxidase in ROS generation. This explanation may be testable by inhibition of xanthine
oxidase in future experiments. Rabbits and humans have little heart xanthine oxidase [17], suggesting that if Kin et al. [1] are correct there might be a good potential for myocardial protection clinically with their interrupted reperfusion approach and also that the rabbit might be a good model for further pre-clinical exploration of their approach, possibly combined with other interventions likely to be protective.

The key practical issue is whether the protection achieved by modifications of early reperfusion conditions has “staying power”. Protection was evaluated by Kin et al. [1] using the well-established tetrazolium staining technique coupled with region-at-risk determination after 3 h of reperfusion. This probably does detect for regional ischemia most of the oncotic cell death at the reperfusion duration examined. However, quantification of apoptotic cell death requires different techniques, and most apoptosis takes much longer than 3 h to be morphologically recognizable [18]. Nevertheless, the irreversible transition to an apoptotic fate may in many cardiomyocytes be triggered by events of early reperfusion, in particular the opening of the mitochondrial transition pore, as has been recently reviewed [19]. In addition, there could be more oncotic cell death beyond 3 h of reperfusion. We suggest further preclinical studies be performed to more fully characterize the protective potential of interrupted reperfusion, both for varying durations of index ischemia and with evaluation following prolonged reperfusion of days duration.

References