Pathogenesis of Myocardial Ischemia-Reperfusion Injury and Rationale for Therapy

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Abstract

Since the initial description of the phenomenon by Jennings \textit{et al} 50 years ago, our understanding of the underlying mechanisms of reperfusion injury has grown significantly. Its pathogenesis reflects the confluence of multiple pathways, including ion channels, reactive oxygen species, inflammation, and endothelial dysfunction. This complexity should not deter our efforts to intervene in this process, however, since nearly 2 million patients annually undergo either spontaneous (in the form of acute myocardial infarction) or iatrogenic (in the context of cardioplegic arrest) ischemia-reperfusion. The purpose of this review is to examine our current state of understanding of ischemia-reperfusion injury and highlight recent interventions aimed at this heretofore elusive target.

Keywords
coronary artery bypass graft surgery; ischemia; myocardial infarction; reperfusion injury

Each year in the United States, there are approximately 1 million myocardial infarctions (MI) and 700,000 patients undergoing cardioplegic arrest for various cardiac surgeries.\textsuperscript{1} Minimizing ischemic time in both of these clinical scenarios has appropriately received a great deal of attention owing to the long-established relationship between duration of ischemia and the extent of myocardial injury. Once coronary flow is restored, however, the myocardium is susceptible to another form of insult stemming from reperfusion of the previously ischemic tissue. Given that cardiac ischemia is either unpredictable (MI) or inevitable (in the operating room), there is great interest in developing strategies to minimize reperfusion-mediated injury.

Historical perspective

The seminal observation that reperfusion following ischemia was associated with myocardial injury was made in 1960 by Jennings and colleagues.\textsuperscript{2} Their report was based on experiments with canine hearts subjected to coronary ligation in which reperfusion appeared to accelerate...
the development of necrosis. For example, the authors noted that the histological changes seen following only 30-60 minutes of ischemia/reperfusion (I/R) were comparable to the degree of necrosis normally seen after 24 hours of permanent coronary occlusion.

Whether reperfusion is independently responsible for tissue injury, or simply hastens the demise of cells otherwise destined for necrosis, remained a matter of debate for some years. Evidence for direct myocardial reperfusion-dependent injury was summarized in 1985 in the classic editorial by Braunwald and Kloner. However, it was not until the discovery of ischemic preconditioning that the independent effects of ischemia and reperfusion began to be unraveled from one another.

In 1986, Murry and Reimer described a process whereby repetitive short bouts of ischemia preceding a prolonged period of ischemia with reperfusion resulted in significantly decreased infarct size in dogs. Subsequently, this “ischemic preconditioning” was confirmed in a number of animal models, including humans, highlighting it as an evolutionarily conserved mechanism. Subsequent experiments revealed that the reperfusion event is key to the initiation of a molecular cascade leading to cardioprotection, thereby serving to solidify the important distinction between ischemia and subsequent reperfusion.

**Mechanisms of I/R injury**

Molecular and cellular events underlying I/R injury are complex, representing the confluence of divergent biological pathways. Further, the extent to which each of these pathways is relevant to human disease remains unclear, as animal models do not always faithfully recapitulate the I/R disease process in humans. These limitations notwithstanding, several key pathophysiologic features of clinically relevant I/R have emerged (Table 1).

Ischemia induces accumulation of intracellular sodium, hydrogen, and calcium ions, culminating in tissue acidosis. Reperfusion, in turn, elicits rapid alterations in ion flux, and some evidence suggests that rapid renormalization of pH paradoxically leads to enhanced cytotoxicity. Sodium-dependent pH regulatory mechanisms, including the Na⁺-H⁺ exchanger and the Na⁺-HCO₃⁻ transporter, are activated, which consequently lead to intracellular sodium accumulation. High sodium concentrations, in turn, drive increases in sarcoplasmic reticular Ca²⁺ via the Na⁺-Ca²⁺ exchange. Enhanced Ca²⁺ entry via sarcolemmal L-type Ca²⁺ channels and a deficient import of cytosolic Ca²⁺ into the sarcoplasmic reticulum by the SERCA Ca²⁺-ATPase further promote Ca²⁺ overload. The result is myofibrillar hypercontractility, ATP depletion, ultrastructural damage to mitochondria, and myocardial stunning.

Cardiac myocytes consume large quantities of energy. To accommodate this requirement, these cells host a high density of mitochondria. Thus, it is not surprising that these complex, energy-generating organelles, filled with reactive intermediates and pro-apoptotic signals, are intimately involved in I/R injury. As part of this, the mitochondrial permeability transition pore (mPTP) has been the center of a growing amount of attention. The inner mitochondrial membrane, responsible for maintaining mitochondrial transmembrane potential, is normally impermeable to ions and proteins. Dissipation of the electrical potential across this membrane is termed “permeability transition”, a process thought to be mediated through the mPTP. Although the constituent protein components of the pore remain unknown, formation of the pore creates a non-selective channel between the inner membrane of the mitochondrion and the sarcoplasm. This results in loss of the electrochemical gradient, release of reactive oxygen species (ROS), and apoptosome formation. Triggers for mPTP include Ca²⁺ overload, rapid normalization of pH, and oxidative stress.
Generation of free radicals through incomplete reduction of oxygen during I/R has been well described. These oxygen species are highly reactive and can quickly overwhelm the cell’s endogenous free radical scavenging system. This, in turn, triggers cellular injury by reactions with lipids, proteins, and nucleic acids. The enzyme, xanthine oxidase, has been particularly implicated as a generator of free radicals in the reperfused heart, as its substrates (xanthine and hypoxanthine) accumulate during ischemia. In addition to damaging nuclear and cytosolic elements, ROS can trigger the opening of the mPTP. This results in a positive feedback loop of additional free radical release from the mitochondria (“ROS-induced ROS release”).

Not only is I/R injury dependent on events occurring within cardiomyocytes, but the endothelium is an active participant as well. The endothelium is the major source of the evanescent molecule, nitric oxide (NO). Under normal conditions, NO generation elicits vasodilation, which has beneficial, protective effects during I/R, likely by influencing oxygen consumption, platelet aggregation, leukocyte adhesion, and free radical scavenging. Paradoxically, in high concentrations, NO may potentiate ROS-mediated toxicity by promoting the formation of highly reactive species, such as peroxynitrite. Beyond NO, the coronary endothelium has several other pathophysiological roles in I/R, such as serving as a source of vasoactive substances and by activating the immune system through expression of cytokines, chemokines, and adhesion molecules.

Recent work has implicated autophagy, an evolutionarily ancient mechanism of controlled cellular cannibalism, in the pathogenesis of I/R. Time will tell whether this mechanism is a suitable target for therapeutic manipulation in this and other heart disease–related contexts.

Endothelial activation and injury increase vascular permeability and recruitment of inflammatory cells. Cellular adhesion molecules elicited by the injured endothelium (eg, ICAM-1, VCAM-1, E-selectin) promote tissue invasion by inflammatory cells. These infiltrating cells, including (and in particular) neutrophils, are directly toxic to the myocardium by secreting proteases, generating ROS, and occluding the microvasculature. Other components of the innate immune system, such as Toll-like receptors, mannose-binding lectin, and the complement cascade, also appear to participate in the pathogenesis of I/R stress. Additionally, there is a growing appreciation of the role of cell-mediated immunity (ie, T-cells and macrophages) in the pathogenesis of myocardial damage after reperfusion.

I/R in acute MI

Although reperfusion injury in the most general sense refers to that component of the infarction process related to restoration of epicardial patency and anterograde blood flow, in the catheterization laboratory, I/R injury is often synonymous with the “no-reflow” phenomenon. The term was first applied to myocardial ischemia following coronary ligation in dogs. Regarded as a dreaded complication of acute MI intervention, it is estimated to occur in more than 30% of cases and is associated with adverse prognosis. No reflow is thought to be related in-part to microvascular plugging by vasoactive debris. While dramatic, no-reflow is probably just the most angiographically apparent form of I/R injury in acute MI, and it should be recognized that significant reperfusion injury occurs even without the obvious “hang-up” of contrast dye.

Deciphering the contribution of I/R to myocardial infarct size in humans is more challenging than in animal models. Acute MI in humans is generally associated with thrombotic occlusion of an epicardial coronary, and this prothrombotic and proinflammatory event is not well captured in models involving surgical ligation of the artery. This may be particularly important as microvascular plugging with leukocytes and platelet “debris” has been implicated as an important component of the I/R process. Another complexity relates to patient comorbidities that influence the myocardial substrate during I/R. Factors such as left
ventricular hypertrophy, diabetes mellitus, and chronic ischemia preceding artery total occlusion (ie, recapitulating ischemic preconditioning), potentially influence sensitivity to I/R injury. The resulting heterogeneity in the human myocardial phenotype renders analyses of experimental models challenging and limits the degree to which the findings can be extrapolated to the human case. Finally, the emergent nature of most acute MI cases (ST-segment elevation MI, in particular) makes it challenging, often both ethically and logistically, to study these patients, as issues arise of informed consent and seeming coercion during the race to achieve vessel patency. Nonetheless, these limitations have not precluded the testing of a series of therapeutic interventions in patients over the last three decades.

**Therapeutic interventions targeting I/R injury in acute MI**

Despite the substantial progress in understanding mechanisms of I/R based on models of acute MI, and the associated enthusiasm for translating these findings into patient care, results of clinical studies have been largely disappointing. Whether this reflects our still incomplete understanding of the biology of I/R, or just a naïve belief that a single intervention could be protective against a process involving multiple major pathophysiological components, is not clear. Initial pilot successes have been met with subsequent failures in larger confirmatory trials. The results of these trials have been summarized elsewhere, but interventions have included a spectrum of targets, including oxidant, inflammatory, sodium-hydrogen exchange, NO metabolism, and metabolic pathways (Table 2).

Despite these setbacks, investigation continues in this field. Erythropoietin (EPO), for instance, is currently undergoing investigation in clinical studies of acute MI following the discovery of EPO receptor expression in the myocardium. EPO has anti-apoptotic activity, positive effects on remodeling, and recruits endothelial progenitor cells. It is hoped that this drug may exert positive effects by one or more of these pathways. Other therapeutic strategies currently in clinical trials include the IL-1-receptor antagonist, anakinra, and glucagon-like peptide-1 analogues.

Although most pharmacologic interventions have been administered systemically, the availability of primary percutaneous coronary revascularization has allowed for direct administration of drug to the coronary endothelium and myocardium. One well-studied drug is adenosine. Adenosine, in addition to its well-known vasodilating properties, is intimately involved with both pre- and post-conditioning (see below), as well as inhibition of mPTP opening. Although systemic adenosine infusion did not reduce overall mortality in the AMISTAD-II trial, some effect on infarct size was noted. Subsequent smaller scale investigation into the potential adjunctive role of intracoronary (IC) administration has demonstrated some benefit with regards to electrocardiographic and angiographic endpoints in the setting of acute MI. Similarly, there has been some enthusiasm, though muted somewhat by the small-scale nature of the studies, for IC nitroprusside in the treatment of no-reflow. This direct NO-donor may be of particular benefit when co-administered with IC adenosine.

Ischemic post-conditioning, a process wherein the myocardium is subjected to repetitive bouts of iatrogenic I/R during the course of an on-going acute MI, is also under clinical investigation. This procedure involves several short intracoronary balloon inflations after primary vessel patency has been re-established. Data from animal models suggest that final infarction size is diminished by this strategy, and some preliminary data in humans appear promising. The mechanism of benefit is unclear, but it may involve induction of a more gradual pH shift in the myocardium or decreasing ROS and Cu²⁺-induced mPTP opening.

There is an evergrowing focus on the role of mPTP in I/R. This structure, as outlined previously, appears to be the common effector of a series of upstream intracellular signals and, therefore,
I/R injury during cardiac surgery

I/R stress resulting from cardiac surgery is distinctly different from that occurring during spontaneous MI. Ischemia is induced artificially by aortic cross-clamping, and myocardial preservation strategies are employed throughout this ischemic period. Cardioplegia is achieved via hyperkalemic, hypothermic cardiac arrest and maintained with the intermittent use of a glucose-containing cardioplegic solution (usually mixed with blood) delivered anterograde in the aortic root and/or retrograde via the coronary sinus. These maneuvers are designed to minimize myocardial metabolic activity and consequent oxygen demand during this period of myocardial vulnerability.

Once surgery has been completed, the aortic cross-clamp is released, and the heart is suddenly and globally reperfused with blood that is fully anticoagulated, immunologically primed by exposure to the cardiopulmonary bypass circuit, and characterized by a very high partial pressure of oxygen. As a result, the post-cardiac surgery myocardium is exposed to dramatic extremes of ischemia and reperfusion. And, again, it is important to recognize that hearts undergoing cardiac surgery are highly heterogeneous, ranging across a spectrum of comorbidities, hypertrophy, and contractile function. Patients undergoing aortic arch surgery may have essentially normal hearts, while other patients may be chronically ischemic with severe contractile dysfunction. These tissue substrates would be expected to react differently to such extremes of environmental stress.

Clinically, I/R injury after cardiac surgery can manifest as arrhythmia, myocardial stunning, low cardiac output, and perioperative myocardial infarction. In patients dying soon after coronary artery bypass surgery (CABG), histologic evidence of I/R on autopsy is detected in 25%-45% of patients. Furthermore, biochemical evidence of myocardial injury (eg, elevated levels of circulating CK-MB and/or troponin) has been clearly linked with adverse events after cardiac surgery.

Interventions targeting I/R injury after cardioplegic arrest

Although the operating theater may appear daunting given the number of personnel, environmental factors, and pieces of equipment needed to successfully manage a patient through cardiac surgery, it is, in many ways, an ideal place to perform research. Duration of ischemia is known, electrolytes and glucose concentrations are meticulously regulated, and hemodynamics can be followed throughout the procedure, including the pre- and post-operative phases. Furthermore, local drug delivery, as opposed to systemic therapy, can be administered reliably via the cannulated coronary circulation.

The cornerstone of cardioprotection in cardiac surgery has been the cardioplegia solution. These solutions typically contain potassium, mannitol, and glucose. By arresting and cooling the heart, the metabolic demands of the myocardium are minimized. However, despite decades of experience with cardioplegia, there has been surprisingly little change in these formulations except for, perhaps, the adoption of blood cardioplegia. And despite the use of cardioplegia, I/R injury still occurs.

There are some small studies supporting the use of metabolic additives, such as pyruvate and glutamate/aspartate. The goal of such metabolic supplementation is to provide the arrested myocardium with energy substrates with favorable characteristics (e.g., avoidance of fatty acid
oxidation), and to replete the cell with anaplerotic substrates. However, evidence to date supporting the use of these agents have not been sufficiently convincing to lead to widespread clinical use.

The concept of ischemic preconditioning has also been applied during cardiac surgery. Either before or just following establishment of cardiopulmonary bypass, the aorta is cross-clamped for several minutes, released for several minutes and then once again cross-clamped for cardioplegia. Several groups have utilized this technique, and some benefit has been seen in small studies. A recent meta-analysis concluded that this form of pre-conditioning decreased post-operative ventricular arrhythmias and inotrope use and translated into shorter ICU stays. These suggestive findings await prospective testing in patients.

Given the association of immune system activation with I/R injury, together with the marked systemic inflammation elicited by surgery and by exposure to the bypass circuit, a number of investigators have focused their attention on modulating immune responses. Leukocyte filters, steroids, and specific volatile anesthetics have all been tested in small studies with no clear signal of benefit. Initial data on the anti-C5a antibody, pexelizumab, appeared promising and prompted two large randomized clinical trials. PRIMO-CABG demonstrated a strong trend toward decreasing death or MI, but these results were not confirmed in the larger follow-up PRIMO-CABG II study. Finally, aprotinin, an antifibrinolytic protein with anti-inflammatory properties, was documented to have favorable effects on myocardial I/R in human and animal studies, but was later taken off the market because of clear evidence for harm associated with its use. In aggregate, results of inhibiting the inflammatory response occurring in cardiac surgery have been disappointing.

The naturally occurring pyridoxine metabolite and purinergic receptor antagonist, pyridoxal-5'-phosphate (MC-1), was studied in two large CABG clinical studies. MC-1 was found to prevent intracellular Ca$^{2+}$ overload and appeared promising for mitigating I/R injury. Indeed, phase II data from the MEND-CABG I study suggested lower post-procedural infarct sizes. Based on this result, a larger phase III study was undertaken. Disappointingly, however, MEND-CABG II failed to find any difference in infarct size and reported a slight early increase in mortality with MC-1 (1.0% vs 0.3%, \(P=0.03\)).

Another strategy to prevent intracellular Ca$^{2+}$ overload is antagonism of the sodium-hydrogen exchanger. Cariporide, a potent inhibitor of this transporter, was first evaluated in the GUARDIAN trial, a catch-all study of patients with non-ST segment elevation acute coronary syndrome (NSTE-ACS) or planned elective revascularization with either percutaneous coronary intervention (PCI) or CABG. An efficacy signal was noted for the CABG subset, and the drug was subjected to a larger trial dedicated to CABG. The primary endpoint in EXPEDITION (death or MI) was lower in the cariporide arm as compared with placebo (16.6% vs 20.3%, \(P=0.0002\)), the first time a phase III clinical study of myocardial protection had met its primary endpoint. However, whereas the composite endpoint was driven by a significant reduction in perioperative MI (18.9% vs 14.4%, \(P<0.0001\)), there was a paradoxical increase in overall mortality (2.2% vs 1.5%, \(P=0.02\)), which appeared to be driven by an increase in cerebrovascular events. Indeed, there was a significant increase in stroke and altered mental status associated with the drug.

Perhaps the most thoroughly studied drug in surgical ischemia-reperfusion is acadesine, a purine analog that increases tissue adenosine levels in energy-deprived tissues. A potent cardioprotective role has been ascribed to stimulation of adenosine receptors in I/R via modulation of mPTP opening. Acadesine, given as an additive in cardioplegia solution, has been investigated in a number of smaller studies, as well as in the ~2,700 patient phase III Acadesine 1024 Trial. Although the largest study failed to show a statistically
significant difference in the primary outcome of peri-procedural MI (3.4% vs 4.0%, \(P=0.24\) in favor of acadesine), a subsequent meta-analysis of all available data on acadesine suggested a 27% reduction in MI (3.6% vs 4.9%, \(P=0.02\)) and a 26% decrease in the combined outcome of stroke/MI/cardiac death (7.6% vs 4.6%, \(P=0.04\)). Acadesine is currently undergoing study in the Reduction in cardiovascular Events by acaDesine in subjects undergoing CABG (RED-CABG) trial, which plans to enroll 7,500 high-risk subjects.

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## Table 1
Pathophysiologic Mechanisms of Myocardial I/R Injury

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Details</th>
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<tbody>
<tr>
<td>Ion accumulation</td>
<td>○ Intracellular calcium overload</td>
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<tr>
<td></td>
<td>○ Increased intracellular sodium</td>
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<td></td>
<td>○ Drop in pH with rapid normalization upon reperfusion</td>
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<tr>
<td>Dissipation of mitochondrial membrane potential</td>
<td>○ Mitochondrial permeability transition pore (mPTP)</td>
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<tr>
<td>Free radical formation/reactive oxygen species (ROS)</td>
<td>○ Generation from Xanthine oxidase</td>
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<td></td>
<td>○ Release of reactive mitochondrial intermediates</td>
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<td></td>
<td>○ Neutrophil infiltration</td>
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<td></td>
<td>○ ROS-induced ROS</td>
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<tr>
<td>Dysregulated nitric oxide (NO) metabolism</td>
<td>○ Loss of NO-vasodilation</td>
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<tr>
<td></td>
<td>○ Accumulation of reactive peroxynitrite</td>
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<tr>
<td>Apoptosis and autophagy</td>
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<tr>
<td>Endothelial dysfunction</td>
<td>○ Cytokine and chemokine signaling</td>
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<tr>
<td></td>
<td>○ Expression of cellular adhesion markers</td>
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<td></td>
<td>○ Impaired vasodilation</td>
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<tr>
<td>Platelet aggregation and microembolization</td>
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<tr>
<td>Immune activation</td>
<td>○ Innate immunity (e.g. complement activation, expression of Toll-like receptors)</td>
</tr>
<tr>
<td></td>
<td>○ Neutrophil accumulation</td>
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<td></td>
<td>○ Cell-mediated damage (macrophage and T-cell)</td>
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</tbody>
</table>
### Table 2

Some notable therapeutic interventions which have been used (with variable success) to mitigate I/R injury in acute MI and CABG

<table>
<thead>
<tr>
<th>Acute MI</th>
<th>Cardiac Surgery</th>
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<tbody>
<tr>
<td><strong>Anti-inflammatory</strong></td>
<td><strong>Metabolic additives in cardioplegic solutions</strong></td>
</tr>
<tr>
<td>○ Inhibition of leukocyte accumulation, e.g. anti-CD11/CD18&lt;sup&gt;125&lt;/sup&gt;</td>
<td>○ Pyruvate&lt;sup&gt;91&lt;/sup&gt;</td>
</tr>
<tr>
<td>○ Complement inhibition, e.g. pexelizumab&lt;sup&gt;126&lt;/sup&gt;</td>
<td>○ Amino acids&lt;sup&gt;92,93&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Increasing local adenosine concentrations</strong></td>
<td><strong>Ischemic pre-conditioning</strong></td>
</tr>
<tr>
<td>○ Systemic infusions&lt;sup&gt;69&lt;/sup&gt;</td>
<td>○ Remote pre-conditioning&lt;sup&gt;124&lt;/sup&gt;</td>
</tr>
<tr>
<td>○ Intracoronary bolus dos&lt;sup&gt;70,71&lt;/sup&gt;</td>
<td>○ Repetitive aortic cross-clamping&lt;sup&gt;94&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Inhibition of mPTP</strong></td>
<td><strong>Volatile anesthetics</strong>&lt;sup&gt;101-103&lt;/sup&gt;</td>
</tr>
<tr>
<td>○ Cyclosporine&lt;sup&gt;85&lt;/sup&gt;</td>
<td><strong>Anti-inflammatory</strong></td>
</tr>
<tr>
<td><strong>Ischemic post-conditioning</strong></td>
<td>○ Leukocyte filters&lt;sup&gt;95-97&lt;/sup&gt;</td>
</tr>
<tr>
<td>○ Repetitive balloon inflations&lt;sup&gt;79-81&lt;/sup&gt;</td>
<td>○ Steroids&lt;sup&gt;98-100&lt;/sup&gt;</td>
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<tr>
<td><strong>Nitric oxide metabolism</strong></td>
<td>○ Complement inhibition&lt;sup&gt;104,105&lt;/sup&gt;</td>
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<tr>
<td>○ Nitroprusside&lt;sup&gt;72-75&lt;/sup&gt;</td>
<td><strong>Prevention of intracellular calcium overload</strong></td>
</tr>
<tr>
<td><strong>Prevention of intracellular calcium overload</strong></td>
<td>○ Pyridoxal-5’-phosphate (MC-1)&lt;sup&gt;110,111&lt;/sup&gt;</td>
</tr>
<tr>
<td>○ Inhibition of Na&lt;sup&gt;+&lt;/sup&gt;-H&lt;sup&gt;+&lt;/sup&gt; exchange, e.g. cariporide&lt;sup&gt;112&lt;/sup&gt;, eniporide&lt;sup&gt;127&lt;/sup&gt;</td>
<td>○ Calcium-channel blockers&lt;sup&gt;133-135&lt;/sup&gt;</td>
</tr>
<tr>
<td>○ Calcium channel blockers&lt;sup&gt;128-130&lt;/sup&gt;</td>
<td><strong>Reducing ROS</strong></td>
</tr>
<tr>
<td><strong>Reducing ROS</strong></td>
<td>○ Inhibition of xanthine oxidase, e.g. allopurinol&lt;sup&gt;131&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Vasodilation</strong></td>
<td><strong>Prevention of intracellular calcium-overload</strong></td>
</tr>
<tr>
<td>○ Potassium-channel opening, e.g. nicorandil&lt;sup&gt;132&lt;/sup&gt;</td>
<td>○ Pyridoxal-5’-phosphate (MC-1)&lt;sup&gt;110,111&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Inhibition of Na⁺-H⁺ exchange, e.g. cariporide\textsuperscript{113,114}

- Increase local adenosine levels
  - Acadesine\textsuperscript{118-122}