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Review Article

Reperfusing the myocardium - a damocles Sword

V.K. Shah[a], K.K. Shalia[b,*]

[a] Sir H.N. Reliance Foundation Hospital and Research Centre, Cardiology Dept. Mumbai 400 004, India
[b] Sir H.N. Medical Research Society, Sir H. N. Reliance Foundation Hospital and Research Centre, Mumbai 400 002, India

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ABSTRACT

Return of blood flow after periodic ischemia is often accompanied by myocardial injury, commonly known as lethal reperfusion injury (RI). Experimental studies have shown that 50% of muscle die of ischemia and another 50% die because of reperfusion. It is characterized by myocardial, vascular, or electrophysiological dysfunction that is induced by the restoration of blood flow to previously ischemic tissue. This phenomenon reduces the efficiency of the present modalities used to combat the ischemic myocardium. Moreover, despite an improved understanding of the pathophysiology of this process and encouraging preclinical trials of multiple agents, most of the clinical trials to prevent RI have been disappointing and leaves us at ground zero to explore newer approaches.

1. Ischemic injury

When blood flow to cardiac myocytes is disrupted by occlusion of a coronary artery, a series of events are set in motion that result in cellular injury and death. This is termed as acute ischemic injury. These events are related to energy production and utilization. During ischemia, anaerobic metabolism prevails, which produces a decrease in cell pH. To buffer this accumulation of hydrogen ions, the sodium/hydrogen (Na^+/H^+) exchanger excretes excess hydrogen ions, which produces a large influx of sodium ions.[1] Ischemia also depletes cellular ATP which inactivates ATPases (e.g., Na+/K+ ATPase), reduces active Ca^{++} efflux, and limits the reuptake of calcium by the endoplasmic reticulum (ER), thereby producing calcium overload in the cell.[2] These changes are accompanied by opening of the mitochondrial permeability transition pore (m-PTP), which dissipates mitochondrial membrane potential and further impairs ATP production.[2] In the heart, these cellular changes are accompanied by activation of intracellular proteases (e.g., calpains) which damage myofibrils and produce hypercontracture and contracture band necrosis (CBN).[3] These phenomena lead to dramatic distortions in myocyte physiology and architecture, including mitochondrial and sarcolemal injury and alterations in intracellular calcium handling. Thus, the four important adverse intracellular events of ischemic injury are (1) rapid normalization of pH, (2) intracellular calcium overload, (3) generation of reactive oxygen species (ROS) and (4) opening of the m-PTP. These alterations and the degree of tissue injury vary in extent with the magnitude of the decrease in the blood supply and with the duration of the ischemic period.[4] Initially, the damage is reversible, and restoration of blood flow during this period recovers normal structure and function. However, if ischemia persists for an extended period of time, this damage becomes irreversible and cell death occurs.

1.1. Reperfusion injury

Although contrary to ischemia wherein anaerobic metabolism prevails, in reperfusion, the supply of oxygen to an ischemic tissue causes similar pathophysiology. Prior to cell death there is a period during which the ischemic myocytes are viable but vulnerable to further injury if blood flow is restored. This results in additional cardiac damage and complications which is referred to as reperfusion injury (RI).[5] It may be viewed as multifactorial responses of the vascular tissue to reflow which causes endothelial dysfunction encompassing vasoactive, thrombotic and inflammatory components.[6–8] RI exacerbates myocardial damage over and above that produced by the initial ischemic insults. These include lethal reperfusion injury, microvascular obstruction (MVO), reperfusion arrhythmias and myocardial stunning.[9]

Reperfusion causes irreversible injury to myocytes which is known as lethal reperfusion injury. Events of ischemia, i.e. an increase of intracellular sodium (Na^+) and opening of m-PTP make the myocardial cells vulnerable to reperfusion. The former i.e.

* Corresponding author at: Sir H.N. Hospital and Research Centre, Sir H.N. Medical Research Society, 4th Floor Court House, L.T. Road, Mumbai, 400 002, India.
E-mail addresses: vkshah45@hotmail.com (V.K. Shah), kavita.shalia@rfhospital.org (K.K. Shalia).

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accumulated Na^+ is normalized by the exchange with Ca^{++} (by sarcolemmal Na^+/Ca^{++} exchanger). This produces calcium overload that triggers uncontrolled hypercontraction and stimulates opening of the m-PTP which further enhances calcium overload. Na^+ together with Ca^{++} accumulation leads to myocyte cell swelling. This contributes to subsequent rupture of the cell membrane when the extracellular osmolality is rapidly normalized by reperfusion.[10] When the blood flow is re-established at the time of reperfusion, sudden reintroduction of molecular oxygen fuels generation of ROS. Opened m-PTPs give an easy access to these ROS to damage mitochondria. Thus, restoration of blood flow to previously ischemic cells results in myocyte hypercontracture and resultant CBN.[11] These leads to significant damage to cytoskeletal elements, ultimately resulting in cell death.

Simultaneously, a massive infiltration of coronary microcirculation by neutrophils and platelets occurs at the time of reperfusion. Reintroduction of neutrophils in post-ischemic myocardium results in their activation, with subsequent adhesion to the endothelial surface and migration in the surrounding tissue. Activated neutrophils, in turn also release oxygen free radicals, proteolytic enzymes, and pro-inflammatory mediators that can directly cause tissue and endothelial damage. Neutrophils also form aggregates with platelets that plug capillaries, thus mechanically blocking the blood flow. Finally, vasoconstrictors released by damaged endothelial cells, neutrophils, and platelets contribute to sustained vasoconstriction of coronary microcirculation, causing no-reflow or MVO. This causes further disruption of microvascular architecture already subjected to endothelial injury induced by ischemia. Although reperfusion removes obstruction to flow in the epicardial circulation, distal flow may remain significantly impaired due to MVO in small arterial vessels.[10,12] The first wave occurs soon after reperfusion and abates within days; the second wave starts later and is associated with healing.[10,11]

Clinical correlates include reperfusion arrhythmias, myocardial stunning and myocytes death.[11] In reperfused ST elevated myocardial infarction (STEMI) patients, the most commonly encountered reperfusion arrhythmias are idioventricular rhythm, ventricular tachycardia and fibrillation.[13] The reversible post-ischemic contractile dysfunction, which occurs on reperfusing the acute ischemic myocardium is referred to as myocardial stunning.[14]

1.2. Identification of reperfusion injury

Identification of patients at highest risk of RI may be an important component of bringing therapies into clinical practice. Recent advances in cardiac magnetic resonance (CMR) imaging have made it possible to retrospectively assess several key features of acute myocardial RI in patients with STEMI who have undergone primary PCI (PPCI) treatment. Performing a CMR scan in the first week following PPCI allows the detection and the quantification of several important prognostic imaging markers including MI size, MVO, and intra-myocardial hemorrhage.[15] There is also the potential to measure myocardial salvage, with the area at risk (AAR) delineated by T2-weighted imaging of myocardial edema.[16] However, this technique for measuring the AAR has its limitations and further study is required to validate its use.[17] A repeat scan performed 4 to 6 months later provides an assessment of final MI size and post-MI LV remodelling in PPCI patients. Therefore, the availability of CMR imaging allows the assessment of the efficacy of therapeutic strategies for preventing acute myocardial RI and provides robust surrogate endpoints of cardio-protection.[18]

Other modalities include, myocardial blush grade (MBG) which has been used as a tool to evaluate myocardial level perfusion, and low blush grades correlate with myocardial dysfunction.[19,20] It is an angiographic index created to assess the status of myocardial reperfusion.[19] It was demonstrated that MBG was a strong angiographic predictor of mortality in patients with thrombolysis in myocardial infarction (TIMI) grade III flow after primary angioplasty.[21] The other tool has been height of the ST segment on epicardial electrocardiograms (ECG).[22] It is utilized as an index of the severity of ischemic injury. ST-segment changes reflect myocardial rather than epicardial flow and hence yield prognostic information beyond that provided by coronary angiogram alone.[22,23] Another measure has been echocardiographic assessment of myocardial perfusion after intracoronary injection of sonicated microbubbles; an investigational technique used to describe myocardial reperfusion in patients with restored patency of the infarct-related coronary artery.[24,25] Such tools may also allow for better clinical assessment of therapeutic effectiveness of drugs tested in clinical trials.

1.3. Prevention of myocardial reperfusion injury

Although the pathophysiology of RI lends itself to potential therapeutic strategies, no treatment directed at RI has been shown to lead to an improvement in clinical outcomes. Potential reasons for the ultimate failure of agents that appeared promising in preclinical trials include (1) the impact of a therapy targeted to a single component of the pathophysiology may be diluted in clinical practice as there are multiple factors contributing to RI, (2) some mechanisms such as neutrophil stimulation may be mediators of injury but also play important roles in the healing process, (3) it may not be possible to administer a therapy at the optimal time in clinical practice (e.g., some agents may perform best if patients are pre-treated), (4) the presence of comorbidities, such as diabetes, hypercholesterolemia, and age, may impact the efficacy of a new treatment. Many trials have been carried out with various modalities for reducing RI in patients presenting with STEMI.[26]

1.3.1. Aspiration thrombectomy prior to coronary stenting

A technique used to overcome reperfusion injury was “Aspiration Thrombectomy Prior to Coronary Stenting” to overcome RI. However, Salloum et al during PCI of Saphenous Vein Grafts had demonstrated that in spite of using distal protection devices i.e. filters to capture the insoluble particulate matter, there were soluble factors which may injure the distal microvascular bed.[27] This may explain the additional myocardial injury occurring post reperfusion therapy. A meta-analysis from combined experience from randomized trials suggested that the use of anti-embolic devices did not decrease early mortality or re-infarction during PCI for native vessel AMI.[28] However, in the subsequent years, the TAPAS (Thrombus Aspiration during Percutaneous coronary intervention in Acute myocardial infarction Study) found that aspiration improved myocardial blush scores and ST-segment resolution (STR), and was associated too with lower mortality at 1 year.[29] Compared with conventional PCI, thrombus aspiration before stenting of the infarcted artery seemed to improve the 1-year clinical outcome after PCI for STEMI.[29] Another meta-analyses showed improved measures of myocardial reperfusion (TIMI flow, myocardial blush, and STR) and procedural outcomes such as reduced no-reflow as well as distal embolization. Three of four trials showed reduced mortality with aspiration thrombectomy which had received a Class IIa indication with primary PCI in the recent ACC/AHA and ESC Guidelines.[30] Unanswered questions include whether there is truly a mortality benefit with aspiration, which subgroups may and may not benefit from aspiration, and whether patients with large thrombus burden are better treated with mechanical thrombectomy.[30] A large and an extended composite endpoint analysis and risk of stroke analyses of the TASTE (Thrombus Aspiration during ST-Elevation myocardial infarction) trial at six month demonstrated no clinical benefit of

routine thrombus aspiration during PCI in patients with STEMI.[31] However, they also concluded that there was no evidence of an increased risk of stroke with thrombus aspiration.[31] In TOTAL (ThrOmbecTomy versus PCI Alone) trial, routine thrombectomy during PPCI did not result in improved MBG or post-PCI TIMI flow grade but reduced distal embolization compared with PCI alone.[32] In this trial distal embolization and not blush grade was independently associated with mortality[32] (Table 1).

1.3.2. Ischemic conditioning

Many interventions to prevent or diminish lethal myocardial RI have been studied.[33] Pre- and post- methods of ischemic conditioning are particularly interesting and have shown some promise, both in preclinical studies as well as in small, but intriguing proof of principle clinical trials. The first is an extension of the principle of cardiac pre-conditioning, in which brief cycles of alternating ischemia and reflow prior to a sustained occlusion reduce the size of the subsequent infarct. The short bouts of ischemic pre-conditioning prior to the induction of lethal ischemia activates cell survival programs that limit post-ischemic injury.[34] It has been observed that this cyclic ischemia can be induced in an organ or tissue other than the heart, yet remain cardio-protective, an intervention termed 'remote ischemic pre-conditioning'.[35,36] Yellon et al[37] have shown that remote ischemic conditioning prior to thrombolysis in STEMI patients reduced myocardial infarct size, making this non-invasive, easily applied low-cost therapy an attractive option in developing nations where health care resources are limited and current therapy is not optimal. The clinical value of ischemic pre-conditioning local or remote is useful only when the timing of the prolonged ischemia such as that induced by cardiac surgery or a PCI is known. It is not applicable to patients with the usual AMI in whom the time when the coronary occlusion will occur is of course not known. However, post-conditioning in which the cyclic periods of ischemia and reflow are begun immediately after the prolonged occlusion is relieved, has also been shown to reduce ischemic injury.[38] and it too can be effective when carried out remotely.[39] Conditioning can also be begun during the occlusion and it is then referred to as per-conditioning[9] (Table 2).

1.3.3. Pharmacological agents used to prevent reperfusion injury (Pharmacologic conditioning)

1.3.3.1. Cyclosporin. The mechanism of protection by Cyclosporin A is that it prevents the opening of the m-PTP.[40] Following encouraging preclinical studies by Griffiths and Halestrap[41] Piot et al[40] conducted a three-center clinical trial and showed that Cyclosporin A reduced infarct size. In this trial, cyclosporin A was infused intravenously just prior to balloon inflation. However, subsequent large randomized trials such as Cyclosporin and Prognosis in Acute Myocardial Infarction Patients (CIRCUS) and Cyclosporine A in Reperfused Acute Myocardial Infarction (CYCLE) have failed to show any benefits.[42,43] The CIRCUS trial randomly assigned 970 patients to an injection of intravenous cyclosporine or placebo before coronary recanalization.[42] There was no difference between the groups in the primary composite outcomes of all cause death such as (1) worsening of heart failure during the initial hospitalization, (2) re-hospitalization for heart failure or (3) adverse left ventricular remodelling at one year (defined as an increase of $\geq$15 percent in the left ventricular end-diastolic volume).[42] The CYCLE trial randomly assigned 410 patients to intravenous cyclosporine A or placebo prior to primary PCI.[43] There was no difference in the rates of the primary end point ($\geq$70 percent ST-segment resolution 60 min after TIMI flow grade III) or secondary end points of high-sensitivity cardiac troponin T on day four, measures of left ventricular function, or clinical events at six-month follow-up[43] (Table 2).

1.3.3.2. Other pharmacological agents used to prevent reperfusion injury. Various pharmacological agents have been tried in animal and human clinical trials which can reduce RI. However, only few have shown some success in clinical trials. Ion channel modulation — Changes in intracellular and extracellular ion concentrations and pH play a role in some of the processes involved in RI. Therefore, ion channels are an attractive target for novel treatments of RI. Among these Potassium-ATP (K-ATP) channel openers are involved in ischemic preconditioning and microvascular vasodilation. E.g. nicorandil, have shown better perfusion and left ventricular wall motion, reduction in adverse events and improved microvascular obstruction by CMR imaging data.[44] Another pharmacological agent, atrial natriuretic peptide (ANP) has shown in animal experimental studies that its prior administration reduced RI through activation of known pro-survival signalling pathways such as cGMP and RISK pathways.[45] In a large clinical trial administering Carperitide (an ANP analogue) at a time of PPCI demonstrated 14.7% reduction in enzymatic mycardial infarct (MI) size.[46] Exenatide is a Glucagon-like peptide-1 analogue, targets prosurvival kinase pathways such as the RISK pathway. Trials have shown 23% reduction in MI size at 3 months by CMR, increase myocardial salvage [47] and 52% reduction in MI size at 1 month by CMR.[48]

The prominent role of oxygen radicals in the pathophysiology of ischemic myocardial damage and reperfusion injury has prompted to evaluate the efficacy of antioxidants and cardio-protective drugs in reducing the damage.[49] The results have been mixed and investigation remains focused at the animal level. A homeostatic balance (proteostasis) between synthesis and

Table 1
Thrombus Aspiration before Stenting of the Infarcted Artery.

No.	Authors/Trials	Year	Outcome
1	Salloum et al. 27,	2005	during PCI of Saphenous Vein Grafts had demonstrated that in spite of using distal protection devices i.e. filters to capture the insoluble particulate matter, there were soluble factors which may injure the distal microvascular bed.
2	Kunadian et al. 28	2007	the use of anti-embolic devices did not decrease early mortality or re-infarction during PCI for native vessel AMI
3	Vlaar et al. 29	2008	TAPAS trial – Compared with conventional PCI, seems to improve the 1-year clinical outcome after PCI for ST-elevation myocardial infarction
4	Brodie et al. 30	2011	Meta-Analysis of 4 trials showed improved measures of myocardial reperfusion (TIMI flow, myocardial blush, and STR) and improved procedural outcomes (reduced no-reflow and distal embolization), and reduced mortality with aspiration thrombectomy
5	Olivecrona et al. 31	2016	TASTE trial demonstrated no clinical benefit of routine thrombus aspiration during PCI in patients with STEMI.
6	Sharma et al. 32	2016	In TOTAL Trial, routine thrombectomy during PPCI did not result in improved MBG or post-PCI TIMI flow grade but did reduce distal embolization compared with PCI alone.

Table 2
Ischemic and Pharmacological Conditioning with Cyclosporin A.

No.	Authors/Trials	Year	Outcome
	Brief cycles of alternate ischemia and reflow		
1	Botker et al. 35	2010	Remote ischaemic preconditioning
2	Yellon et al. 37	2015	
3	Zhao et al. 38	2003	Post Conditioning-Cardiac
4	Kerendi et al. 39	2005	Remote Post Conditioning
5	Yellon and Hausenloy. 9	2007	Per-conditioning
	Pharmacological Conditioning: Cyclopsorin A		
1	Piot et al. 40	2008	Cyclosporin A reduced infarct size
2	Cung et al. 42	2015	CIRCUS Trial –failed to show the benefit
3	Ottani et al. 43	2016	CYCLE Trial-failed to show the benefit

degradation of defective proteins is crucial to maintain proper health of the dynamically and metabolically active cardiomyocytes. This balance depends not only on oxidative stress but also with reductive stress in the myocardium and subsequent posttranslational modification of vital and sensitive cardiac proteins that are involved in the basic function of contractile myocytes. Hence the drugs that need to be developed to treat the cardiovascular pathologies like ischemia/reperfusion injury should be able to modulate both oxidative and reductive stress pathways. Nevertheless, a series of mitochondria targeted antioxidants developed recently provides further impetus to delve further to characterize their pharmacokinetics and pharmacodynamic properties and progress with the pharmaceutical development.[50] One of the pharmacological target tried successfully in animals is Melatonin.[51] It is an indolamine, principally produced by the pineal gland with a circadian rhythm and it regulates various physiological and neuroendocrine functions through specific receptors or directly in subcellular organelles.[51] Their actions include as anti-oxidant and as multiple immune-modulator at both the cellular and humoral levels.[52,53] Table 3 lists various other pharmacological agents that have shown success in animal studies but no effect in human clinical trials. In addition, pharmacological agents that have been tried in animal studies and await human trials are also listed in Table 3 (http://www.uptodate.com/contents/reperfusion-injury-of-the-heart).

1.4. Therapeutic hypothermia and hyperoxemia

Myocardial metabolism can be decreased at lower temperatures (32–33 °C) affording cardio-protection analogous to cold cardioplegia used by cardiac surgeons during coronary bypass grafting. Cardio-protection involves limiting MI size, reducing the inflammatory response, decreasing platelet aggregation and thus increasing myocardial efficiency. In animal models of acute coronary occlusion, hypothermia induced before reperfusion reduced infarct size. Endovascular coils and external cooling blankets have been used to lower core temperature during PCI for acute myocardial infarction.[54] However, the CHILL-MI trial, which used rapid infusion of cold saline and endovascular cooling prior to PCI, failed to demonstrate reduction in the primary end point of infarct size.[55] In another prospective, multicentre, randomized controlled pilot trial of peritoneal hypothermia (to cool to 34.7 °C in patients with STEMI), showed no effect on primary end point of MI size (CMR at 3–5 days).[56]

Hyperoxemia/Hyperbaric oxygen reduces MI sizes by decreasing tissue edema, reducing formation of lipid peroxide radicals, altering nitric oxide synthase expression, inhibiting leukocyte adherence and plugging in the microcirculation.[57] In AMIHOT I[57] and AMIHOT II[58] trials, infusion of super saturated O_2 within 6 h of STEMI, resulted in significant reduction in the infarct size with non-inferior rates of major cardiovascular events at 30 days.

Table 3
Latest Pharmacological Agents under Study Used to Conquer Reperfusion Injury (http://www.uptodate.com/contents/reperfusion-injury-of-the-heart).

Pharmacological Agents	Human Clinical Trial
Adenosine	A substrate for adenosine triphosphate (ATP) replenishment, causes vasodilation, platelet and neutrophil inhibition. No significant difference between patients undergoing elective PCI with intracoronary Adenosine or Placebo PREVENT-ICARUS Trial
MTP-131	A cell-permeable peptide that enhances mitochondrial energetics and improves myocyte survival during reperfusion. In a Phase 2 clinical trial, intravenous MTP-131 or placebo following successful PCI with stenting, showed no significant difference in infarct size between the two groups.
Intravenous sodium nitrite	In experimental models, sodium nitrite reduces ischemic reperfusion injury to the heart. NIAMI trial, intravenous infusion of sodium nitrite showed no improvement.
Losmapimod	Mitogen-activated protein kinase (MAPK) is a stress-activated kinase expressed in the myocardium and endothelial cells that regulates cellular responses, including contraction and death. An oral inhibitor of p38 MAPK; Losmapimod; lowered inflammation but not protection against serious adverse events in phase 2 SOLSTICE trial.
Inhibitors of delta-protein kinase C	Protein kinase C isoenzymes modulate myocardial protection. PROTECTION AMI trial, three doses of delcasertib; inhibitor of the delta isoform of protein kinase C or placebo given intravenously showed no difference in infarct size.
Vasodilators	Several members of the sydnonimine class of nitric oxide (NO) donors have reduced infarct size in an animal model. Vasodilators such as Papaverine demonstrated success in improving TIMI flow grades in epicardial arteries; but caused ventricular arrhythmias. Pharmacological Agents Tested only in Animal Models so far
P2Y12 inhibitors	Platelet activation contributes to microvascular injury and reperfusion injury in acute MI. Cangrelor in preclinical studies significantly ameliorated infarct size when administered prior to the onset of reperfusion
Glycoprotein IIb/IIIa inhibitors	Potent inhibitors of platelet activity that improve outcomes in acute MI and part of the success of the intracoronary glycoprotein IIb/IIIa inhibitor administration in PCI may be also due to its effect on leukocyte integrin receptors, which can also be tested for reduction in reperfusion injury. Other components under investigations shown to reduce reperfusion injury are Erythropoietin, estrogen, heme oxygenase-1, and hypoxia induced factor.

However, there was no difference in 14 day MI size assessed with SPECT.

2. Conclusion

Myocardial reperfusion reduces ischemic cell death nevertheless injures the surviving myocardium. In the 1960s, well before the first human reperfusion studies were carried out, Jennings et al[59] and Krug et al[60] demonstrated impaired reperfusion after release of a temporary coronary occlusion. Kloner et al[61] reported that reperfusion caused microvascular damage with swelling of capillary endothelial cells and myocytes, leading to what was termed the 'no reflow phenomenon'. Areas of no-reflow have been found to be associated with infarct expansion in animals and a high mortality in patients.[62] Indeed, in 1985, myocardial reperfusion was referred to as 'a double-edged sword.[63] Since then many methods or components demonstrated hope in pre-clinical studies but failed at clinical trials.[64–66] Still a lot needs to be done. It has been estimated that timely reperfusion can salvage approximately 50% of the severely ischemic myocardium from necrosis. Prevention of lethal myocardial RI should salvage additional 40%.[67] If the latter is successful, it would further substantially reduce the mortality of AMI, a goal still to be reached.

Conflicts of interest

None.

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