

New therapeutic targets for myocardial infarction

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Coronary artery disease (CAD) is the most common cause of morbidity and mortality worldwide and is projected to be the leading cause of death and disability worldwide by 2020. CAD contributes 30.9% of global mortality and 10.3% of global burden of diseases. In the Indian population, incidence of CAD among the younger generation is quite high (up to 12–16%) compared to the Western population (5%). Sedentary and stressful lifestyle has been suggested as an important risk factor for CAD, along with the conventional risk factors such as hypertension, diabetes mellitus, hypertriglyceridaemia, high levels of LDL-cholesterol, central obesity and low levels of HDL-cholesterol. CAD is characterized by insufficient blood supply to regions of the myocardium, which can lead to development of necrosed zones that are ultimately replaced by fibrous tissue. Reperfusion is one of the mechanisms of restoring blood supply to ischaemic myocardium and limiting myocardial damage. However, it is associated with cell death which further increases complications such

as diminished cardiac contractile functions and arrhythmias. The phenomenon is named as ischaemia–reperfusion injury. Although therapy for CAD has entered a new era, most of the current therapies were developed in the absence of defined molecular targets. Increasing knowledge of the biochemical and cellular alterations occurring in myocardial infarction (MI) has led to the development of novel and potentially more effective therapeutic approaches to treat the disease. The role of peroxisome-proliferator-activated receptors (PPARs) and K_{ATP} channel blockers in the treatment of MI has been documented. However, many promising molecules, especially the anti-apoptotic drugs, phosphodiesterase-5 inhibitors are yet to be approved due to safety issues. Meanwhile, two targets – gene therapy and inhibitors of Na^+/H^+ exchanger have emerged as validated targets for the pharmacotherapy of MI. The present review discusses the different functional targets which are currently being used or bear potential as treatment for MI.

Keywords: Coronary artery disease, ischaemia–reperfusion, myocardial infarction, oxidative stress, therapeutic targets.

MYOCARDIAL ischaemia (MI) is the result of insufficient blood flow to the heart tissue, usually due to narrowing or obstruction in the lumen of a coronary artery. If blood flow to the myocardium is severely compromised or protracted, irreversible changes may occur in the ultra structure of the heart tissue. In addition, reperfusion of the myocardium following an ischaemic event may induce injury to the heart tissue from the release of reactive oxygen metabolites, liberation of pro-inflammatory mediators and release of intracellular calcium. MI can be subcategorized on the basis of anatomic, morphologic and diagnostic information. From an anatomic or morphologic standpoint, there are two main types of MI: trans-mural and non-trans-mural. A trans-mural MI is characterized by ischaemic necrosis of the full thickness of the affected muscle segment, extending from the endocardium to epicardium. A non-trans-mural MI is defined as an area of ischaemic necrosis that does not extend through the full thickness of the myocardial wall segment. In a non-

trans-mural MI, the area of ischaemic necrosis is limited to both the endocardium and the adjacent myocardium. The endocardial and subendocardial zones of the myocardial wall segment are the least perfused regions of the heart and are most vulnerable to conditions of ischaemia. Injury obtained from both the ischaemic event and the reperfusion (R) periods is intimately interwoven together. MI/R injury represents a clinically important sequel of events that may arise during various instances like revascularization of artery, at the time of cardiac surgery when blood flow through the coronary vasculature is interrupted and other pathologic events such as shock and cardiac arrest¹. As mentioned above, depending upon the duration and severity of the cardiac insult, the ensuing myocardial injury can be reversible (myocardial stunning) or irreversible (myocardial necrosis or infarction). Myocardial injury can also be characterized by various abnormalities, including the development of arrhythmias, contractile dysfunction, ultrastructural damage as well as defects in intracellular biochemical homeostasis¹.

The treatment for MI/R injury is still a major concern. A number of pharmacological approaches have been proposed, with limited success, to delay myocardial necrosis and decrease reperfusion injury. These approaches include the use of beta-blockers, ACE inhibitors, antiplate-

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let agents, thrombolytics, calcium channel blockers, nitrates, antioxidants and free-radical scavengers. However, these agents are not effective. Therefore, recently, attention has been focused on new therapies that prevent/attenuate ischaemia and reperfusion injury associated with MI. Two potential strategies for protecting the heart are ischaemic preconditioning (IPC) and ischaemic postconditioning (IPost), which describe the cardioprotection obtained from applying transient episodes of MI and reperfusion, either before or after the index ischaemic event respectively.

Ischaemic preconditioning

IPC is an adaptation by the myocardium and is defined as the phenomenon where a transient ischaemic insult, not long enough to cause permanent damage, paradoxically increases tolerance to subsequent, more prolonged ischaemia. IPC protects the heart against post-infarction left-ventricular dysfunction and ventricular arrhythmias. Ischaemia-induced activation of both adenosine, α adrenergic receptors and opening of ATP-sensitive potassium channels and stress protein synthesis are thought to be the potential mechanisms of this phenomenon. Nonetheless, exploration of the exact mechanisms of IPC might improve the treatment strategies for acute myocardial infarction in the future².

Ischaemic postconditioning

Postconditioning reduces the oxidant-induced injury. Moreover, it attenuates the local inflammatory response to reperfusion. According to the more recent literature, short bouts of ischaemia after an index ischaemia can also initiate cardioprotection, e.g. improve post-ischaemic dysfunction or reduce infarct size, which has been called postconditioning. IPC and IPost follow the common signal transduction pathway at the time of myocardial reperfusion, the pharmacological manipulation of which has the potential to generate new strategies for treatment of MI³.

Emerging biological targets for MI

Gene therapy for MI

Gene therapy can be defined as the treatment or prevention of disease by introduction of recombinant DNA into the heart tissue. The normal myocardium and the ischaemic reperfused myocardium are proven targets for the delivery of DNA and viral vectors. These strategies make it possible to treat MI/R injury using gene therapy and are a good potential therapeutic target in the future.

Heat shock protein genes: Temperature and shock increase the expression of certain genes that encoded for a special group of proteins referred to as heat shock proteins

(HSP). According to their molecular weight, HSPs are divided into five groups or families. (i) HSP 20–30 kDa, (ii) 50–60 kDa families. (iii) HSP 70 kDa family, a major group of HSP that acts as a molecular chaperone. This family of HSP is associated with protein synthesis. Over-expression of these HSPs protects cells against heat stress and hypoxia. (iv) HSP 90 kDa family. (v) HSP 100–110 kDa family. This family of proteins is considered necessary for an organism to survive severe stress conditions.

The function of HSP70 gives us some clues as to the mechanisms by which these proteins may induce myocardial protection. During ischaemia, the cellular internal milieu changes profoundly with intracellular accumulation of protons and sodium ions. These changes are compounded by an increase in free radicals and a marked increase in intracellular calcium associated with reperfusion. Under these circumstances, the tertiary structure of proteins may change sufficiently to alter their function. In the presence of excess HSP70, either adverse conformational changes are prevented or re-assembly of denatured proteins is promoted⁴. More and more evidences suggest that HSPs play a direct role in myocardial protection from ischaemia and reperfusion injury⁵. Currie *et al.*⁶ first described the association between HSP and myocardial protection. They showed that raising the body temperature of rats from 37°C to 42°C for at least 15 min increased both cardiac inducible HSP and catalase activity after 24 h. During this time, the heart became more resistant to ischaemia and reperfusion injury. Almost any kind of stress, including hypoxia, ischaemia and oxidative stress can rapidly induce expression of HSP genes.

HSP72 and exercise: Walsh *et al.*⁷ first described that exercise induced the release of HSP72. It was demonstrated that 60 min of running exercise at 70% of maximal oxygen uptake resulted in a marked increase in the circulating level of HSP72. Collectively, these studies demonstrated that physical exercise results in an increase in the systemic HSP72 concentration and that this increase is not the result of tissue damage. The underlying mechanism suggested that during exercise, sympathoadrenally innervated tissues may be stimulated to release HSP72, which activates the specific components of the immune system. This suggests that HSP gene therapy may become a novel method for myocardial protection from MI/R injury through endogenous protective mechanisms.

Bcl-2 gene: Bcl-2 is the original member of a growing family of cell-death regulators. Bcl-2 displays a unique oncogenic function that promotes cell survival, rather than cell proliferation. The molecular weight of Bcl-2 is approximately 25 kDa. The intracellular Bcl-2 protein extends cell survival because it specifically blocks apoptotic cell death following a variety of signals such as hypoxia and ischaemia. Although Bcl-2 is not constitutively expressed in the heart, MI/R induces Bcl-2 production in the heart.

Apoptosis may be the predominant form of cell death after coronary artery ligation in the rats, followed by necrosis at later time points. Coronary artery ligation also induces up-regulation of Bcl-2 expression in the zone bordering viable cells. Sawa *et al.*⁸ have also shown that hearts transected for over-expression of Bcl-2 manifested less apoptosis and improved recovery of left ventricular end-diastolic pressure, as well as less enzyme leakage after MI/R injury. How Bcl-2 prevents myocytes from apoptotic cell death is not well understood. Bcl-2 may inhibit cell death by acting as either an antioxidant or a cellular pro-oxidant, triggering enhanced expression of cellular antioxidant defence mechanisms. Findings from these studies suggest that *Bcl-2* gene therapy might be an effective treatment target for MI/R injury in the future.

Anti-apoptotic drugs

Apoptosis is a highly regulated form of cell death, essential to the normal development of an organism and designed to eliminate unwanted cells. It is a distinct form of cell death in which the cells commit suicide, which leads to elimination of cells without inducing an inflammatory response. It is characterized by cell shrinkage, chromatin condensation, DNA fragmentation, membrane blabbing and formation of apoptotic bodies⁸. In recent years, accumulating evidence from both experimental as well as clinical studies indicates that cardiomyocytes undergo apoptosis in several cardiovascular diseases, including ischaemic heart disease (IHD), heart failure and atherosclerosis⁹.

Induction of apoptosis in ischaemic reperfused hearts has been suggested to be mediated by a variety of pathways which may or may not be interrelated, including, activation of Fas (cell surface receptor protein of the TNF receptor family that induces apoptosis) or tumour necrosis factor- α (TNF- α) receptors, activation of p53 and c-Jun kinase pathways, down-regulation of anti-apoptotic Bcl-2 protein, up-regulation of pro-apoptotic Bax protein, and infiltration and activation of neutrophils and/or macrophages. These pathways are more likely to be involved during reperfusion, whereas ischaemia itself may initiate apoptosis via a mitochondrial pathway. Mitochondria play multiple roles in apoptosis, which includes supplying ATP that is necessary for execution of apoptosis, releasing cytochrome *c* and apoptosis-inducing factor proteins that are involved in caspase activation and nuclear fragmentation.

At its simplest, the apoptotic machinery includes three basic components: death proteases (caspases), CED-4/Apaf-1 adapter molecules and Bcl-2 family members. Caspases represent the executionary arm of the apoptotic machinery. CED-4/Apaf-1 appears to play a critical role in the conversion of pro-caspases into active caspases through direct physical interactions¹⁰. The Bcl-2 family includes both pro- and anti-apoptotic members that can regulate each other to shift the balance from a pro-

apoptotic environment to an anti-apoptotic environment, and vice versa. 1,4 Dihydropyridine calcium channel blockers like amlodipine and nifedipine reduce excessive apoptosis and give cardioprotection.

Low molecular weight caspase inhibitors may represent a new class of drugs for the treatment of acute cardiovascular diseases in which apoptosis is believed to play a significant role. A better understanding of the role of apoptosis in MI/R injury will probably lead to the discovery of additional targets for pharmacological intervention.

Inhibitors of Na^+/H^+ exchanger

The Na^+/H^+ exchanger (NHE) is involved in intracellular pH homeostasis of many mammalian cell types. Till date, seven NHE isoforms (NHE1–NHE7) have been identified. NHE1 is the most predominant isoform expressed in the heart, where it contributes to cardiomyocyte pH homeostasis. Extensive pre-clinical studies have indicated that NHE inhibitors afford substantial protection in different animal models of myocardial ischaemia and reperfusion, but the results of clinical trials involving eniporide and cariporide were mixed. Nevertheless, an excessive stimulation of NHE results in an increase of intracellular Na^+ concentration and a subsequent activation of Na^+/K^+ ATPase, with a consecutive increase in energy consumption¹¹. The high intracellular Na^+ level activates the sarcolemmal $\text{Na}^+/\text{Ca}^{2+}$ antiporter systems that result in raised intracellular Ca^{2+} . At the cardiac level, this intracellular Ca^{2+} overload subsequent to NHE-1 activation is involved in I/R injuries like MI, myocardial stunning and tissue necrosis¹². Extensive pre-clinical studies indicated that NHE inhibitors afford substantial protection in animal models of myocardial ischaemia and reperfusion injury. The cardioprotective effect of (S)-T-162559 and zoniporide, specific NHE1 inhibitors has been shown to be more potent than eniporide and cariporide respectively, in a rabbit model of I/R injury¹³.

Potassium ATP channels

It has been hypothesized that potassium ATP channels couple myocardial metabolism to membrane electrical activity and suggested that the opening of K_{ATP} channels may serve as an endogenous cardioprotection mechanism¹⁴. Indeed, a number of K_{ATP} channels openers have produced myocardial protection in numerous model of ischaemia and two K_{ATP} channels, antagonist 5-hydroxy-decanoate (5-HD) and glibenclamide were found to block the protection given by K_{ATP} channels openers¹⁵. As one of the mechanisms for cardioprotection by enhancing the cardiac action potential duration by accelerating phase-3 repolarization, which would inhibit calcium entry into the cell via L-type channels and prevent calcium overload. In addition, membrane hyperpolarization or slowing of de-

polarization would also inhibit calcium entry and slow down or prevent the reversal of the sodium–calcium exchanger that normally extrudes calcium in exchange for sodium. The result of these actions would be a reduction in calcium overload during ischaemia and possibly early reperfusion with subsequent increased cell viability.

Potassium-channel openers (PCOs) are drugs that activate (open) ATP-sensitive K^+ channels in vascular smooth muscle. Opening of these channels hyperpolarizes the smooth muscle, which closes voltage-gated calcium channels and decreases intracellular calcium; this leads to relaxation and vasodilation. Several PCOs, viz. nicorandil, pinacidil and monoxidil have been extensively screened in experimental studies and found to be effective in acute coronary diseases. Great expectations are awaited with the use of PCOs as adjuncts for preconditioning in patients with acute coronary syndromes.

Phosphodiesterase 5 inhibitors

Phosphodiesterase 5 (PDE5) inhibitors have transformed the treatment of erectile dysfunction (ED). Vascular endothelial provides a link between ED and cardiovascular diseases. It plays a vital role in the regulation of circulation, and it is now recognized that vascular disease is the major cause of ED. Recent studies suggested that sildenafil has additionally preconditioning-like powerful cardioprotective effect in the animal models of MI/R injury¹⁶. Sildenafil has been found to provide cardioprotection through nitric oxide generated from endothelial and/or inducible nitric oxide synthases and opening of mitochondrial ATP-sensitive potassium channels. Future demonstration of the cardioprotective effect in patients with the relatively safe and effective FDA-approved PDE-5 inhibitors, such as sildenafil, vardenafil and tadalafil, could have an enormous impact on bringing the long-studied phenomenon of ischaemic and pharmacologic preconditioning to the clinical forefront. Mechanistic studies suggest that these drugs provide cardioprotection through NO generated from eNOS/iNOS, activation of protein kinase C/ERK signalling and opening of mitochondrial ATP-sensitive potassium channels.

Peroxisome-proliferator-activated receptors modulators

The peroxisome-proliferator-activated receptors (PPARs) are a subfamily of the 48-member nuclear receptors superfamily and regulate gene expression in response to ligand binding¹⁷. Various fatty acids serve as endogenous ligands for PPARs, whereas some members of the superfamily (farnesoid receptor) bind with bile acids while others (liver X receptors) bind with oxysterols. PPARs regulate gene transcription by two methods. (i) Trans-activation, which is DNA-dependent and involves bind-

ing to PPAR response elements of target genes and heterodimerization with the retinoid X receptor. (ii) Trans-repression, which may explain the anti-inflammatory actions of PPARs. It involves interfering with other transcription-factor pathways in a DNA-independent manner¹⁸. The nuclear receptor family of PPARs was named for the ability of the original member to induce hepatic peroxisome proliferation in mice in response to xenobiotic stimuli. However, studies on the action and structure of the three human PPAR isotypes (PPAR α , PPAR δ and PPAR γ) suggested that these moieties are intimately involved in nutrient sensing and regulation of carbohydrate and lipid metabolism. PPAR γ is principally involved in cellular assimilation of lipid via anabolic pathways. Thiazolidinediones like pioglitazone and rosiglitazone are positive modulators of PPAR γ , while fibrates such as fenofibrate, bezafibrate, ciprofibrate and gemfibrozil act as PPAR α agonists. In general, PPAR α activation enhances free fatty acid oxidation, controls the expression of multiple genes regulating lipoprotein concentration and has anti-inflammatory effects.

Poly-ADP ribose polymerase inhibitors

PARP also known as poly (ADP ribose) synthetase, is an abundant nuclear enzyme of eukaryotic cells that participates in DNA repair in response to genotoxic stress. PARP gives rise to energy-consuming cycles by transferring ADP ribose units from NAD^+ to nuclear proteins. This process results in rapid depletion of the intracellular NAD^+ and ATP pools, slowing down the rate of glycolysis and mitochondrial respiration, eventually leading to cellular dysfunction and death¹⁹. Over-activation of PARP represents an important mechanism of tissue damage in various pathophysiological conditions associated with oxidant stress, including MI/R. Activation of PARP importantly contributes to the development of endothelial dysfunction in streptozotocin-induced model of diabetes in mice²⁰.

Thiemermann *et al.*²¹ and Cuzzocrea *et al.*²² independently demonstrated that pharmacological inhibition of PARP reduces myocardial necrosis and improves cardiac function in coronary artery ischaemia–reperfusion injury. PARP catalyses an energy-consuming polymerization of ADP-ribose, resulting in NAD depletion, inhibition of glycolysis and mitochondrial respiration, and the ultimate reduction of intracellular high-energy phosphates in the reperfused heart. Therefore, inhibition of PARP activity prevents energy depletion and granulocyte inflammation. It has been demonstrated *in vitro* in cardiac myoblasts, endothelial cells and vascular smooth muscle cells that PARP has a significant role in cell injury induced by peroxynitrite, a potent oxidant species produced in various forms of reperfusion. PARP inhibitors, PJ34 (*N*-(6-oxo-5,6-dihydro-phenanthridin-2-yl)-*N,N*-dimethylacetamide, 3-aminobenzamide and nicotinamide improve myocardial

and endothelial function. Furthermore, the pharmacological blockade of PARP also results in protection against peroxynitrite injury in cardiomyocytes and reduces myocardial infarct size in regional ischaemia models²³.

Most PARP inhibitors compounds fall into the categories of monoaryl amides and bi, tri or tetra cyclic lactams. Recently, several other classes of more potent and selective PARP inhibitors have been synthesized and which act as competitive inhibitors of the enzyme, i.e. the inhibitors block NAD⁺ binding to the catalytic domain of the enzyme.

Adenosine receptor modulator

Adenosine is a vasodilator that plays an important role in the regulation of coronary circulation. However, it also promotes glycolysis and activates K_{ATP} channels. Adenosine also strongly inhibits neutrophil function, such as superoxide anion production, protease release and adherence to coronary endothelial cells. Hence adenosine attenuates ischaemic injury as well as neutrophil-mediated reperfusion injury. Adenosine has also been implicated in the cardioprotective phenomenon of IPC. However, recent basic and clinical studies have shown that adenosine and its analogues may serve as potent cardioprotective agents in the clinical setting of acute MI. The underlying mechanism of adenosine exhibiting cardioprotection is due to inhibition of neutrophil aggregation, as well as adherence of neutrophils to endothelial cells²⁴, which subsequently reduce ATP depletion during ischaemia and improve repletion of ATP on reperfusion, stimulate myocardial glycolysis and normalize the oxygen supply/demand ratio through their vasodilating and anti-adrenergic properties²⁴. Recent studies using adenosine analogues suggest that drugs which selectively target adenosine A₂ receptors at reperfusion can protect the heart from MI/R injury in a wide variety of animal models.

Norton *et al.*²⁵ reported that CGS21680 given at the time of reperfusion significantly reduces infarct size in rabbit heart. Similarly, Xu *et al.*²⁶ found that AMP579 attenuated both production of reactive oxygen species (ROS) at reperfusion and cell death via activation of A₂ receptor. However, the role of A₂ receptor in suppression of ROS is still ambiguous.

Oxidative stress and antioxidants

The biological importance of ROS has attracted enormous interest during recent years due to their major role, both beneficial and noxious, in numerous vital processes. The main mechanism responsible for production of ROS is the respiratory chain²⁷, in particular its complex I and III. Several strategies are used for production from ROS. One of them is to increase glutathione content by *N*-acetyl-

cysteine and CoA by pantothenic acid. Several antioxidants such as ascorbic acid, α -tocopherol, α -lipoic acid and curcumin²⁸ have demonstrated cardioprotective action in different animal models of MI.

Probucol is a lipid-soluble, cholesterol-lowering drug with potent antioxidant properties that has been shown to protect myocardial function and improve endogenous antioxidant function. The study suggested that chronic use of probucol once in MI induced heart failure, results in improved haemodynamics and endothelial function²⁹.

Aldose reductase inhibitors

Aldose reductase (AR) is a monomeric, NADPH-dependent enzyme that is a member of the aldo-keto reductase family. This enzyme catalyses the reduction of aldo sugars and other saturated and unsaturated aldehydes, and constitutes the first step of the polyol pathway. AR has been implicated in the etiology of diabetes complications, atherosclerosis, and MI/R injury. Since the activity of AR was higher in ischaemic hearts, lactate/pyruvate ratio, a measure of cytosolic redox state (NADH/NAD⁺), has been shown to increase during ischaemia³⁰. Inhibition of AR with zopolrestat attenuated the increases in lactate/pyruvate ratio and cytosolic redox state. The decrease in lactate/pyruvate ratio by AR inhibitors has conserved NAD⁺ and aids glycolysis by reducing NAD⁺ used by the sorbitol dehydrogenase system.

Further cellular metabolic pathways demonstrate that AR could affect substrate metabolism in response to ischaemia. Pharmacological inhibition of AR increased myocardial glycolysis and glucose oxidation, as well as conserved ATP during ischaemia. These demonstrate that ischaemia increases myocardial AR activity and pharmacological inhibitors of AR may prove as a potential therapeutic adjunct with mainstream therapies for myocardial infarction.

Antiplatelet therapy

Antiplatelet agents are used to reduce the risk of major cardiovascular events in various settings. Antiplatelet monotherapy reduces the ischaemic events by 25% as compared to placebo³¹. Aspirin, clopidogrel, ticlopidine, aspirin in combination with clopidogrel, and dipyridamole are effective in preventing recurrent vascular events among various subgroups of patients with vascular disease. Aspirin, clopidogrel or their combination should be used to treat patients with acute ST-elevation MI (STEMI) and non-ST elevation MI (NSTEMI)³¹. Many clinical trials with their results published recently, have supported the therapeutic efficacy of antiplatelet therapy, particularly with the use of aspirin, clopidogrel, ticlopidine and GpIIb/III_a inhibitors (e.g. abciximab) in patients with acute coronary syndrome^{32,33}. Thus use of antiplatelet

agents for cardiac failure is well established and supported by clinical evidences, and is a part of the treatment regimen.

Beta-adrenergic blockers

A great deal of interest has been generated on the use of beta-adrenergic antagonists in the treatment of acute MI and in the prevention of recurrences for those who have survived an initial attack. Beta-adrenergic receptor blocking agents are competitive antagonist at β_1 and β_2 or both types of adrenergic receptors. Beta-receptor antagonists reduce sinus rate, decrease the spontaneous rate of depolarization of ectopic pacemakers, slow down conduction in the atria and in the AV node, and increase the functional refractory period of the AV node³⁴.

Timolol, metoprolol, atenolol and propranolol have been shown to exert cardioprotective effects. Carvedilol is a beta-adrenergic antagonist with vasodilatory properties (alpha1-antagonism), and has been extensively evaluated in the treatment of patients with heart failure. In patients with chronic heart failure, carvedilol improves left-ventricular (LV) ejection fraction over 6–12 months of treatment, and attenuates LV remodelling³⁵.

Current literature advocates their beneficial effects in patients with heart failure though; the underlying mechanisms apart from beta-adrenergic receptor blockade are still being explored. Beta-adrenergic blockers seem to have a promising future as a therapy for MI.

Endothelin receptor antagonists

Endothelin-1 (ET-1), one of the strongest vasoconstrictive peptides, is constrictively secreted by vascular cells and cardiomyocytes. This release is increased in various pathophysiological conditions, including MI/R injury, hypertension and CHF³⁶. There are three isoforms of endothelin (ET), ET-1, ET-2 and ET-3. ET-1 is the most abundant in the cardiovascular system, and acts on two distinct receptors, ET-A and ET-B. However, the role of each subtype has not been completely clarified.

ET receptor blockade has been shown to have therapeutic potential in experimental and early clinical studies of hypertension, atherosclerosis and heart failure. The non-selective ET-A receptor antagonists are bosentan, CGS-27830 and TAK-044, while selective ET-A receptor antagonists are BMS-20794, BQ-123 and several other compounds. In patients with severe congestive heart failure, acute infusion of the nonselective antagonist bosentan increased cardiac output and reduced systemic and pulmonary resistance.

C1-esterase inhibitor

C1-esterase inhibitor (C1-Inh) therapy was introduced in clinical medicine about 25 years ago, as replacement

therapy for patients with hereditary angioedema caused by deficiency of C1-Inh. Accumulating evidence suggests that administration of C1-Inh may have beneficial effect in acute MI³⁷. Activation of complement, contact and coagulation system in acute MI has been observed that leads to increase in vasoactive peptides such as bradykinin or the anaphylatoxins³⁷. C3 is common to all the pathways of complement activation and augments myocardial cell injury and apoptosis during HI/R, whereas complement system inhibition with C1Inh, a serine protease inhibitor, exerts markedly cardioprotective effects³⁷.

Nuclear factor kappa B

Nuclear factor kappa (NF-kappaB) regulates the expression of several genes involved in inflammation, immune responses, apoptosis, cell survival and proliferation. Many of these genes are activated during reperfusion injury. Activation of NF-kappaB is dependent of phosphorylation of its inhibitor, Ikappa Balpha, by the specific inhibitory kappa B kinase (IKK) subunit, IKKbeta. Bay 65-1942, an ATP-competitive inhibitor that selectively targets IKKbeta kinase activity, has demonstrated to reduce acute myocardial damage following MI/R injury³⁸.

Several studies have demonstrated that NF-kappaB is substantially involved in the progression of cardiac remodelling. However, it remains uncertain whether the continuous inhibition of NF-kappaB is effective for the prevention of myocardial remodelling. IMD-0354, a novel phosphorylation inhibitor of IkappaB that acts via inhibition of IKKbeta, inhibits fibrosis, accumulation of macrophages, and expression of several factors (transforming growth factor-beta1, monocyte chemoattractant protein-1, matrix metalloproteinase-9 and -2) in the non-infarcted myocardium. In conclusion, inhibition of NF-kappaB activation may reduce the pro-inflammatory reactions and modulate the extracellular matrix and provide an effective approach to prevent adverse cardiac remodeling after MI.

Anti-TNF-alpha therapy

TNF was initially discovered as a result of its antitumour activity and has been shown to mediate tumour initiation, promotion and metastasis. In addition, dysregulation of TNF-alpha has been implicated in atherosclerosis and myocardial infarction. TNF-alpha, however, is a critical component of effective immune surveillance and is required for proper proliferation and function of NK cells, T cells, B cells, macrophages and dendritic cells.

Myocardial necrosis induces complement activation and free-radical generation, triggering a cytokine cascade initiated by TNF-alpha release. Clinically, serum levels of TNF-alpha are increased after myocardial infarction and after cardiopulmonary bypass. TNF-alpha, once blocked

by specific inhibitors like TNF- α blockers (infliximab, etanercept) should profoundly decrease cardiovascular risk³⁹.

Glucagon-like peptide 1

Glucagon-like peptide 1 (GLP-1), a gut incretin hormone that stimulates insulin secretion, also activates anti-apoptotic signalling pathways such as phosphoinositide 3-kinase and mitogen-activated protein kinase in pancreatic and insulinoma cells. The GLP-1 receptor is a G protein-coupled receptor and is a distinct member of the glucagon–secretin receptor superfamily that has been shown to function by causing intracellular calcium influx in addition to up-regulating cAMP. Interestingly, cAMP has been demonstrated to protect against apoptosis in several cell types other than myocardial. In isolated cardiac myocytes, GLP-1 has also been shown to elevate cAMP in addition to demonstrating positive chronotropic and inotropic effects. However, GLP-1 could directly protect the heart against such injury via kinase signalling pathways. Administration of GLP-1 improved left ventricular function in patients with acute MI and left ventricular dysfunction⁴⁰.

Insulin-like growth factor 1

Insulin-like growth factor-1 (IGF-1), the primary mediator of growth hormone effects, is an important regulator of cell growth, differentiation and apoptosis. IGF-I deficiency is known to be associated with premature atherosclerosis and increased mortality from cardiovascular diseases. Recent evidence suggests that cardiovascular disease risk may also be elevated among apparently healthy individuals who have serum IGF-I levels in the low-normal range⁴¹.

Accumulating evidence has indicated that IGF-1 plays a specific role in the cardiovascular system, in addition to its well-established growth promoting and metabolic effects. IGF-1 promotes cardiac growth, improves cardiac contractility, cardiac output, stroke volume and ejection fraction. In humans, IGF-1 improves cardiac function after MI by stimulating contractility and promoting tissue remodelling. Furthermore, IGF-1 facilitates glucose metabolism, lowers insulin levels, increases insulin sensitivity and improves the lipid profile. These suggest that IGF-1 is an attractive therapeutic potential for MI.

Protein kinase C

Molecular cloning studies have indicated that protein kinase C (PKC) exists as a family of at least 12 distinct isoforms. The conventional PKC isoforms α , β and γ contain Ca^{2+} -binding domain which requires Ca^{2+} for activation. Cardiac PKC activity has been observed to increase

in pressure-overloaded cardiac hypertrophy and heart failure in animals and human studies. In addition, varying degrees of changes in PKC activities have been observed in cardiac dysfunction due to diabetes. Chronic AT1-receptor antagonism is capable of protecting the heart against myocardial infarction in a PKC-epsilon-dependent way. Furthermore, chronic treatment with ACE-I is suggested to have suppressing effects on IPC, possibly caused by reduced PKC-epsilon expression⁴².

Translocations of specific PKC isoforms are believed to participate in several functions, including the opening of mitochondrial K_{ATP} channels. It is now evident that epsilon and delta PKC have distinct temporal and opposing roles in regulating myocardial damage induced by MI/R. Activation of epsilon PKC before ischaemia protects the heart by mimicking preconditioning, whereas inhibition of delta PKC during reperfusion protects the heart from reperfusion-induced damage.

Conclusion

Myocardial infarction has been one of the leading causes of mortality and morbidity worldwide for several decades. Various pathways have been explored and identified to be involved in the development and precipitation of MI, thus making it a multi-faceted disease. The present article is an attempt to provide a brief review of these pathways, among which some have been well correlated to MI and are being exploited as therapeutic interventions for it, like use of beta-blockers, antiplatelet agents, NHE, inhibitors, PPAR modulators and anti-TNF- α agents, whereas other targets like gene therapy, anti-apoptotic agents, potassium ATP channel openers, PDE5 inhibitors, PARP inhibitors, adenosine receptor modulators, endothelin receptor antagonists, C1-esterase inhibitors, NF-kappaB antagonists, glucagon-like peptides, insulin-like growth factors and PKC inhibitors have been identified as possible therapeutic targets by preliminary investigational studies. Further detailed studies are required to document their efficacy and safety.

MI being a disease of the developed and developing nations, constantly attracts researchers to identify all possible approaches to find its most suitable cure, which will eventually bring out newer mechanisms and targets. This article is an attempt to compile such targets available in the literature till date, but the list may go on increasing with continuous efforts in MI research.

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