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Review Article

Cellular and molecular mechanisms of endothelial ischemia/reperfusion injury: perspectives and implications for postischemic myocardial protection

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Abstract: Ischemia/reperfusion (I/R) injury is a major cause of myocardial damage. Despite continuous efforts, minimizing I/R injury still represents a great challenge in standard medical treatments of ischemic heart disease, *i.e.*, thrombolytic therapy, primary percutaneous coronary intervention, and coronary arterial bypass grafting. Development of effective interventions and strategies to prevent or reduce myocardial I/R injury is therefore of great clinical significance. Endothelial dysfunction plays a significant role in myocardial I/R injury, which renders endothelial cells an attractive target for postischemic myocardial protection. The rapidly evolving knowledge of the mechanisms of endothelial I/R injury helps broaden perspective for future development of novel strategies targeting endothelium for alleviating myocardial I/R damage. This review provides a comprehensive summary of the cellular and molecular mechanisms of endothelial I/R injury. Current perspectives and future directions for developing endothelium targeting therapeutics for postischemic myocardial protection are further discussed.

Keywords: Endothelium, gap junction, ischemia/reperfusion, ion channels, microRNA

The vascular endothelium is a single layer of cells that lines the entire circulatory system. By counteracting leukocyte adhesion and platelet aggregation to prevent inflammation and thrombosis and actively regulating vascular tone with the production of vasoactive substances, endothelial cells play a key role in maintaining vascular health. Disturbance of functional integrity of endothelium, known as "endothelial dysfunction", represents a complex pathophysiological entity including inflammatory activation and perturbation of anticoagulatory properties as well as abnormal vasomotion [1]. Endothelial dysfunction significantly contributes to the pathogenesis of a variety of cardiovascular disorders including myocardial ischemia [2].

Ischemic heart disease is the most common cause of myocardial ischemia. Previous studies have demonstrated the pivotal role of endothelial dysfunction in the initiation and progression of this disease [3, 4]. Moreover, strong associations have been reported between endothelial dysfunction and a number of well-defined risk factors for ischemic heart disease such as smoking, hypertension, obesity, and diabetes [5]. Myocardial ischemia is inevitable in cardiac surgery requiring cardiopulmonary bypass. The no- or low-reflow phenomenon after myocardial ischemia/reperfusion (I/R) resulting from endothelial edema, neutrophil and platelet plugging, microthrombosis, and enhanced vasomotor may lead to inadequate coronary perfusion that further compromises cardiac function [6].

Cellular and molecular mechanisms of endothelial dysfunction in myocardial I/R

I/R induces vascular endothelial dysfunction through multiple mechanisms including cytotoxicity caused by pH change, oxidative stress

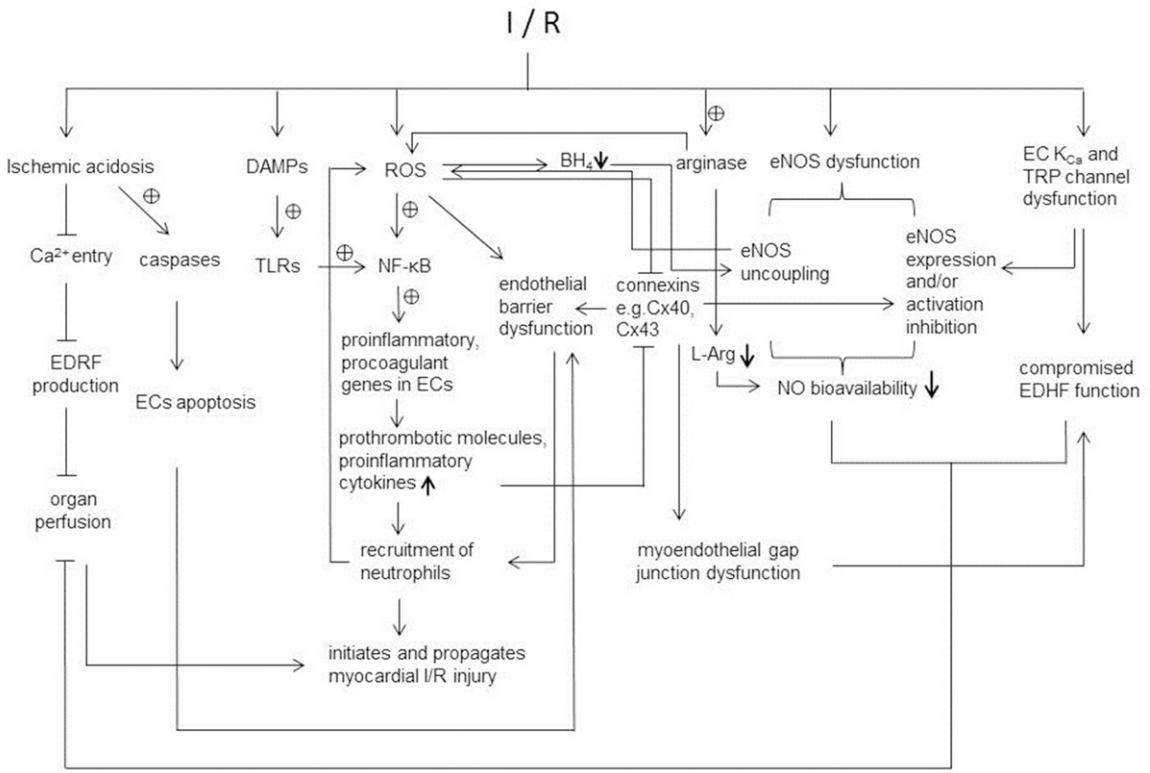

Figure 1. Schematic diagram summarizing the mechanisms and significance of endothelial dysfunction in myocardial I/R injury. EC: endothelial cell, eNOS: endothelial nitric oxide synthase, EDHF: endothelium-derived hyperpolarizing factor, EDRF: endothelium-derived relaxing factor, NO: nitric oxide, ROS: reactive oxygen species, DAMPs: damage-associated molecule patterns, BH_4: tetrahydrobiopterin, L-Arg: L-arginine, NF-κB: nuclear factor kappa-B, TLRs: Toll-like receptors, K_{Ca}: Ca^{2+}-activated K^+ channels, TRP: transient receptor potential channels.

resulting from overproduction of reactive oxygen species (ROS), and endothelial nitric oxide synthase (eNOS)-nitric oxide (NO) inhibition, etc [7, 8]. Studies in recent years provided new insights into the molecular mechanisms of endothelial I/R injury such as modulation of ion channels and gap junction proteins.

The role of acidosis-induced cytotoxicity in ischemic endothelial damage was evidenced by ischemic acidosis-induced activation of caspases, *i.e.*, caspase-12 and caspase-3, in endothelial cells of coronary arteries [9]. By upregulation of the antiapoptotic protein Bcl-xL, acidic preconditioning protects coronary endothelial cells from ischemic apoptosis [10]. In addition, extracellular acidosis strongly suppresses Ca^{2+} entry into endothelial cells thereby inhibiting the production of vasoactive substances, which may also be involved in I/R-induced endothelial dysfunction [11].

ROS is abundantly generated by cardiomyocytes, coronary vascular endothelium, and inflammatory cells during I/R through incomplete reduction of O_2 in which xanthine oxidase, NADPH oxidase, NO synthase (unconjugated), cyclooxygenase, and lipoxygenase may all be involved [8]. Activation of endothelial cell by oxidative stress promotes intravascular microthrombosis, reduction of blood flow and activation of inflammatory cells. Expressions of E-selectin, P-selectin, and intercellular adhesion molecules (ICAMs) on the surface of activated endothelial cells promote the recruitment of neutrophils, the principal effector cells of inflammation during I/R [12]. Nuclear factor kappa-B (NF-κB) plays a key role in I/R-induced endothelial cell activation. Tyrosine phosphorylation of IκBa induced by oxidative stress results in the dissociation of this inhibitory protein from NF-κB, leading to the nuclear translocation of NF-κB and subsequent activation of transcription of proinflammatory, procoagulant, and vasoactive genes expressed in endothelial cells, which consequently initiates and propagates myocardial I/R injury [13]. Oxidative stress may also activate mitogen-activated pro-

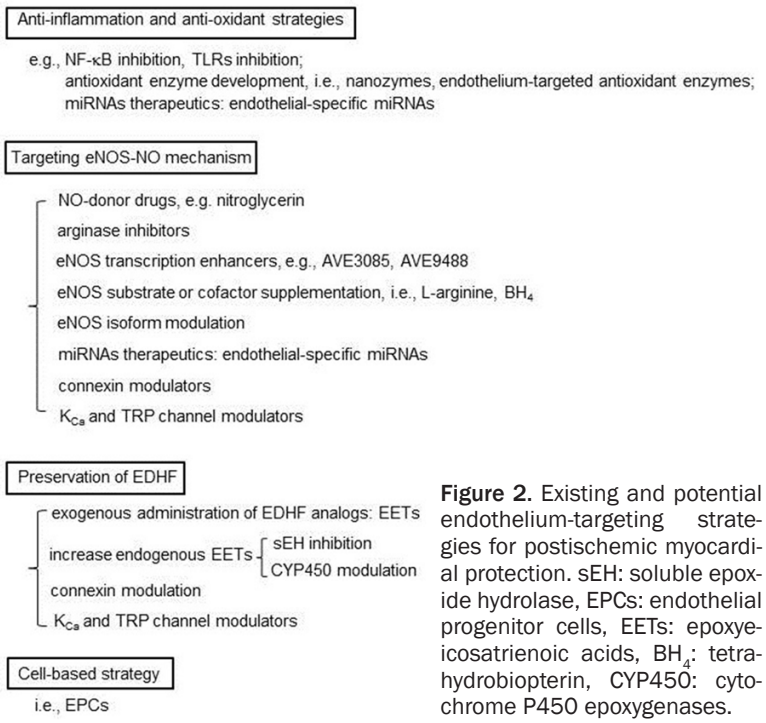

Figure 2. Existing and potential endothelium-targeting strategies for postischemic myocardial protection. sEH: soluble epoxide hydrolase, EPCs: endothelial progenitor cells, EETs: epoxyeicosatrienoic acids, BH_4: tetrahydrobiopterin, CYP450: cytochrome P450 epoxygenases.

tein kinases (MAPKs) that are capable of phosphorylating NF-κB subunits to modulate transactivational activity of NF-κB [14]. In addition to be a target of ROS, endothelial cells are also an important source of ROS. ROS generated by endothelial cells through xanthine oxidase, NADH/NADPH oxidase, and uncoupled eNOS significantly contributes to vascular dysfunction after I/R that involves acceleration of NO inactivation [15].

Endothelial permeability increases following myocardial I/R. The loss of barrier function of endothelial cells can be attributed to ROS released from activated leukocytes that cause changes in endothelial cytoskeletal structures and promote the formation of intercellular gap [16]. Activation of endothelial contractile machinery due to cell re-energization as well contributes to endothelial barrier failure [17]. Endothelial barrier dysfunction consequently promotes migration of neutrophils and other inflammatory cells into the injured myocardial tissue and further potentiates I/R injury.

Moreover, I/R disrupts the balance between endothelium-derived constricting and relaxing factors thus interrupts blood flow and organ perfusion. I/R increases the production of vasoconstrictors such as endothelin-1 [18]. A considerable body of evidence suggests the significance of reduction of endothelium-derived relaxing factors, in particular, NO and endothelium-derived hyperpolarizing factor (EDHF) in the disturbance of blood flow in myocardial ischemia and related conditions [19-24].

In addition to its potent vasodilatory effect, NO inhibits platelet aggregation and leukocyte adhesion as well as vascular smooth muscle proliferation to act as an important component of the endogenous defense mechanism against vascular injury, inflammation, and thrombosis. The decrease of NO bioavailability is a well-known consequence of myocardial I/R. Multiple mechanisms including eNOS inhibition [25, 26], arginase activation [27, 28], and increased production of ROS [29] are involved in I/R-induced NO loss through reduction of production and/or acceleration of inactivation. Inhibition of store-operated Ca^{2+} entry by acidosis results in decreased production of NO, which may also contribute to endothelial dysfunction during ischemic assault [11]. In fact, in an in vitro I/R model, measurement of NO by using a NO micro sensor provided a direct evidence of the decrease of NO in coronary arteries after hypoxia/reoxygenation (H/R) exposure [30].

Uncoupling of eNOS is another mechanism by which myocardial I/R compromises eNOS-NO function. Instead of producing NO, uncoupled eNOS becomes a source of ROS generation [31]. This functional switch of eNOS occurs when substrate L-arginine or cofactor tetrahydrobiopterin (BH_4) is insufficient, which in myocardial I/R can result from arginase activation that increases the consumption of L-arginine, and ROS production (particularly peroxynitrite $ONOO^-$) that leads to oxidization and degradation of BH_4 [32]. Reduction of NO and production of $O_2^{\cdot-}$ worsen endothelial I/R injury. Moreover, NO and $O_2^{\cdot-}$ can affect primary contractility of actin-myosin fibers within myocytes, putatively via effects on Ca^{2+} storage in the sar-

coplasmic reticulum. Diminished myofiber contraction resulting from NO inhibition and $O_2^{\cdot-}$ overproduction significantly affects cardiac output [33].

Contribution of EDHF in vasodilatation increases as vessel size decreases [34, 35], which highlights the significance of EDHF in blood flow regulation. Opening of intermediate and small conductance Ca^{2+}-activated K^+ channels (IK_{Ca} and SK_{Ca}) on the plasma membrane of endothelial cells underlies the classical EDHF pathway [36]. The mechanisms include channel opening-induced conductible hyperpolarization via myoendothelial gap junctions and K^+ efflux-mediated hyperpolarization by activation of inwardly rectifying K^+ (Kir) channels and Na^+-K^+-ATPase on adjacent smooth muscle cells [37]. In some vasculature including coronary arteries, non-classical EDHF response mediated by epoxyeicosatrienoic acids (EETs) may also exist. EETs not only activate endothelial IK_{Ca} and SK_{Ca} but also open myocyte large-conductance K_{Ca} (BK_{Ca}) to relax vessels [38]. Although potentiation of the EDHF-type response was reported in animal models of myocardial I/R and cerebral I/R [39, 40], which supports the "compensatory or backup" theory of EDHF-mechanism in conditions involving NO loss, contradictory evidence shows compromised EDHF function under I/R conditions. For example, in porcine coronary arteries exposed to H/R, the EDHF-mediated relaxation was significantly attenuated [23, 41, 42]. H/R also blunted the EDHF-response in coronary microveins [24]. Further membrane potential measurement showed a decrease of hyperpolarization mediated by EDHF in smooth muscle cells of coronary vasculature [43]. Furthermore, we recently demonstrated that H/R inhibits IK_{Ca} and SK_{Ca} currents in coronary endothelial cells and the inhibition of IK_{Ca} and SK_{Ca} activity underlies the impairment of EDHF responses caused by H/R [43].

As discussed above, the mechanisms of endothelial dysfunction in myocardial I/R injury are summarized in **Figure 1**.

Significance/potential of endothelial protective strategies in myocardial I/R injury

To date, I/R injury still remains a major challenge in standard medical treatments of ischemic heart disease, *i.e.*, thrombolytic therapy and primary percutaneous coronary intervention [44], and in open heart surgery. Myocardial I/R induces coronary endothelial dysfunction that in turn promotes myocardial injury. Exaggerated inflammatory reactions following endothelial cell activation are closely associated with oxidative stress during myocardial ischemic assault, which rationalizes the traditional antiinflammatory and antioxidant strategies for endothelial and myocardial protection. Postischemic cardiac performance may benefit from well-preserved coronary blood flow by strategies protecting endothelial dilatory function, *i.e.*, NO and EDHF pathways. New approaches targeting cellular mechanisms underlying these endothelium-derived relaxing factors have the potential to become new treatments for myocardial ischemia.

Anti-inflammation and antioxidant strategies for cardioprotection

Cardioprotection conferred by interventions targeting neutrophil influx, such as neutralization of P-selectin or depletion of neutrophil has been reported in ischemic myocardial injury [45, 46]. Administration of monoclonal antibody against leukocyte adhesion molecule CD18 (ligand for ICAM-1) protects coronary endothelium and myocardium in neonatal lamb hearts following cardioplegic arrest, evidenced by preserved coronary blood flow and better recovery of left ventricular developed pressure [47]. Inflammatory reactions resulting from endothelial cell activation can be suppressed by NF-κB inhibition. Transfection of NF-κB decoy oligonucleotides into isolated rat heart blocked ICAM-1 upregulation and inhibited neutrophil adhesion to small coronary venules [48]. The dramatic increase of NF-κB in patients undergoing heart surgery with cardioplegic intervention [49] added clinical evidence supporting the potential of NF-κB inhibition in postischemic myocardial protection.

There is increasing evidence suggesting the role of Toll-like receptors (TLRs) in endothelial activation associated with myocardial I/R injury. As a key component of the innate immune system, TLRs induce both innate and adaptive inflammatory responses. TLRs are also expressed on non-immune cells including cardiomyocytes and vascular endothelial cells. Recent studies demonstrated that activation of TLR2 and TLR4 in endothelial cells by damage-asso-

ciated molecule patterns (DAMPs), i.e., heat shock protein 27 (HSP27) released from ischemic/reperfused myocardium leads to NF-κB activation and upregulates endothelial expression of monocyte chemoattractant protein (MCP)-1 and ICAM-1 production [50]. TLR2 and TLR4 signaling were observed to mediate leukocyte migration and postischemic vascular permeability [51]. Considering the role of activation of conventional TLR-NF-κB pathway in immune cells, cardiomyocytes, and endothelial cells in the pathogenesis of myocardial I/R injury, development of pharmacological interventions that interfere with the expression and/or activity of TLRs signaling may lead to new treatments of myocardial ischemia.

It has to be mentioned that although cardioprotective effect of antiinflammatory strategies has been shown in a number of animal experimental studies, clinical trials aiming to inhibit inflammation however yielded unsatisfactory results, suggesting that inflammation is not solely an injurious process, but also mediates processes essential for proper tissue healing. Therefore, balancing the inflammatory forces between damage and repair needs to be emphasized in future development of antiinflammatory strategies, such as strategy targeting endothelial cell activation, for cardioprotection against I/R injury.

Endothelium-dependent vasodilator responses of coronary arteries were better preserved after cardiac arrest using cardioplegic solution containing inhibitors of hydroxyl radical synthesis, *i.e.*, deferoxamine or manganese superoxide dismutase (SOD) [52]. Inclusion of organic antioxidants such as ascorbate and deferoxamine in St Thomas' Hospital cardioplegic solution improved the recovery of aortic flow in rat heart after global ischemic arrest [53]. The protective effect of antioxidants on endothelium involves the inhibition of ROS-induced endothelial cell activation and NO inactivation [54, 55]. As the role of enzyme sources of endothelium-derived ROS become clear, it is possible to develop more specific therapies targeting endothelial redox mechanisms for myocardial protection. Meanwhile, recent advances in enzyme engineering such as nanozyme and cell-targeting delivery approaches help enhance the efficiency of antioxidant enzymes for endothelial protection. Benefit from its permeation-enhancing activity, pluronic based polymer nanoparticles containing catalase and SOD ("nanozyme") show remarkable protective effect against I/R in a transient middle cerebral artery occlusion model [56]. SOD conjugated with antibodies to endothelial surface marker molecule, i.e., platelet endothelial cell adhesion molecule 1 (PECAM-1) provides targeted delivery of SOD into endothelial cells, which quenches endothelial ROS and affords superior anti-inflammatory effects compared with untargeted SOD formulations in vascular endothelium, associated with a tangible therapeutic benefits in an animal model of ischemic stroke [57].

Significance of targeting eNOS-NO mechanism in cardioprotection

The significance of NO in inhibiting neutrophil accumulation, inactivating superoxide radicals, and improving coronary blood flow establishes the role of this intracellular signaling molecule in myocardial protection. Moreover, NO was found to mediate the cardioprotective effect of a number of clinically used strategies such as preconditioning and postconditioning [58], which further supports the concept of targeting eNOS-NO mechanism for myocardial protection under I/R conditions.

Early attempts to enhance NO function include application of NO precursor L-arginine or NO donors such as nitroglycerin. Administration of these agents or supplementation in cardioplegia preserves postischemic endothelial function in both animals and humans and improves postischemic ventricular performance [59-63]. In fact, the use of NO-donor drugs is considered an effective replacement therapy in "NO-deficient" disorders. However, the reduced responsiveness to nitrovasodilators, caused by nitrate resistance and nitrate tolerance, yet remains a problem to be solved.

Strategies targeting mechanisms by which I/R inhibits NO function were further developed, including inhibition of arginase activation [28], restoration of eNOS down-regulation [26], and modulation of eNOS uncoupling [64]. Use of arginase inhibitor restored the NO-mediated function in I/R vessels [28]. Addition of eNOS-transcription enhancer AVE3085 in St. Thomas' Hospital cardioplegia was observed to restore NO production suppressed by H/R and protect coronary dilator responses [26]. Experimental studies in cultured bovine aortic endothelial cells demonstrated that exogenous BH_4 supplementation during oxidative assault prevents

eNOS uncoupling and increases NO production [32]. Further, in a co-culture system of cardiomyocytes and endothelial cells, increasing BH_4 content in endothelial cells by either pharmacological or genetic approaches was able to reduce the susceptibility of cardiomyocytes to H/R injury [65].

Recent studies demonstrated that human eNOS gene is subject to alternative splicing and the expression of splice variants, *i.e.*, eNOS13A, produce truncated proteins lacking the reductase domain with no eNOS activity. Moreover, eNOS13A forms heterodimers with full-length eNOS and such heterodimerization significantly reduces eNOS activity [66, 67]. These findings suggested that regulation of eNOS activity via modulation of the expression of eNOS isoforms could be of potential therapeutic interest in cardiovascular disorders including myocardial I/R injury in which endothelial dysfunction plays a role in the pathogenesis.

Cardioprotective potential of EDHF preservation

Preservation of EDHF component can be achieved by several approaches that have been proven effective in experimental studies. Addition of $EET_{11,12}$, a possible chemical analogue of EDHF to cardioplegic solutions protects endothelial function of coronary arteries with restoration of EDHF-mediated responses [68, 69], which can be explained by the direct "EDHF mimetic" effect of $EET_{11,12}$. Interestingly, a recent study in an *in vivo* rat model of infarction demonstrated that administration of EETs prior to ischemia activates eNOS and increases NO production [70], which provided a new insight into cardioprotective mechanisms of EETs [71]. In addition to exogenous administration of EET analogs, approaches aiming to increase the endogenous concentration of EETs also show therapeutic potential in myocardial ischemia that include inhibition of soluble epoxide hydrolase (sEH) [72] to suppress EETs metabolism and overexpression of cytochrome P450 epoxygenases (CYP450) to increase EETs production [73].

Cardioprotective potential of targeting gap junctions

Gap junctions formed by connexins (Cx) play an important role in cell-cell communication and homeostasis in various tissues including vasculature, which enable a direct passage of ions, metabolites, or electrical signals from one cell to another. Electrical coupling along the endothelium and between endothelium and smooth muscle is central in arteriolar conducted response and control of vascular resistance. The vascular gap junctions are assembled from one or more of four connexin proteins: Cx37, Cx40, Cx43, and Cx45. Cx40 and Cx43 are expressed in both endothelial and smooth muscle cells while Cx37 is typically confined to endothelium and Cx45 locates at smooth muscle [74]. Endothelial expression of Cx40 is influenced by various factors such as oxidative stress, pro-thrombotic molecules, pro-inflammatory cytokines, and classical cardiovascular risk factors [75, 76]. A recent study in a clinically relevant setting of I/R injury showed that the expression of Cx40 disappears from the endothelium in the infarct zone and in mice with endothelial-specific deletion of Cx40, infarct size increases after I/R. The cardioprotective effect of endothelial Cx40 in cardiac I/R injury was suggested to be associated with a decrease in neutrophil infiltration through ecto-5'-nucleotidase/CD73-dependent regulation of vascular cell adhesion molecule-1 (VCAM-1) expression at the surface of endothelial cells [77-79]. Consistently, in a hindlimb ischemic model, Cx40 deficient animals exhibited profound and rapid failure of ischemic limb survival [80]. Studies in a monolayer of cultured microvascular endothelial cells showed that hypoxia followed by abrupt reoxygenation reduces interendothelial electrical coupling via oxidant- and PKA-dependent signaling that targets Cx40, which provided a mechanistic explanation for the compromised arteriolar function following H/R [81]. Considering that eNOS and Cx40 can exist in a complex and endothelial Cx40 expression is essential for proper expression and function of eNOS [82, 83], I/R/H/R-induced Cx40 modulations are therefore expected to result in functional changes of eNOS-NO pathway.

Direct electrical communication between endothelial and smooth muscle cells via myoendothelial gap junctions plays an essential role in EDHF signaling, which further reveals the relevance of connexin proteins to the endothelial control of vascular tone. Blockade of myoendothelial gap junctions with mimetic peptides specifically against Cx37, Cx40 and Cx43 has been

observed to prevent endothelium-dependent subintimal smooth muscle hyperpolarization [84, 85]. Rapid endothelial cell-selective loading of Cx40 antibody also blocked EDHF-type signaling [86].

Given the important role of gap junctions in conducting vasodilator responses, manipulation of connexin function and/or expression may represent a potential approach for tackling endothelial dysfunction. The improvement of vasorelaxation in response to preconditioning was demonstrated to be associated with increases of Cx40 and Cx43, as well as a more efficient gap junction coupling in endothelial cells [87]. However, successful translation of these basic scientific discoveries into clinical application will require further studies and future developments of selective pharmacological tools that allow targeting gap junctions in a connexin-isoform and cell type-specific manner.

Cardioprotective potential of targeting endothelial ion channels

Endothelial ion channels, in particular, Ca^{2+}-permeable channels, *i.e*, transient receptor potential (TRP) channels [88], and K^+ channels, *i.e*, IK_{Ca} and SK_{Ca}, emerge as promising therapeutic targets for endothelial I/R injury. An increase of $[Ca^{2+}]_i$ in endothelial cells is required for the activation of NO generating enzyme eNOS [89]. Opening of IK_{Ca} and SK_{Ca} or/and production of EETs that underlie the EDHF action also depend on endothelial $[Ca^{2+}]_i$ rise [36, 90]. On the other hand, membrane hyperpolarization of endothelial cells resulting from IK_{Ca} and SK_{Ca} opening in turn enhances driving force for Ca^{2+} entry, promoting Ca^{2+} influx and NO production [91]. These lines of evidence suggest the significance of Ca^{2+}-permeable and K^+ channels and the functional interplay between these two distinct types of channels in the modulation of endothelial function.

IK_{Ca}/SK_{Ca} and TRP channels were found to be affected by I/R and hyperkalemic exposure, which provided scientific basis for targeting these channels during cardiac surgery for endothelial protection. In coronary arteries exposed to H/R, pharmacological activation of IK_{Ca} and SK_{Ca} channels improves EDHF-responses including relaxation and hyperpolarization [43]. The potential of IK_{Ca}/SK_{Ca} activators in the treatment of cardiovascular disorders through the improvement of endothelium-derived hyperpolarizations and NO-mediated function was discussed in depth in recent review articles [92, 93].

I/R/H/R affects TRP channels, *i.e*., TRPC3 and TRPV4, and associated vascular endothelial function. Through inhibiting membrane translocation of the channel, H/R suppresses TRPC3 channel activity and Ca^{2+} influx via TRPC3 in coronary endothelial cells, resulting in reduction of NO production. Activation of TRPC3 channels restores NO production in coronary arteries subjected to H/R [30]. Most recently, we demonstrated that supplementation of the TRPC3 channel activator in hyperkalemic cardioplegia such as St Thomas' Hospital and Histidine-Tryptophan-Ketoglutarate solutions preserves TRPC3-mediated Ca^{2+} influx in endothelial cells and improves EDHF-mediated relaxation of coronary arteries [94]. In a mice model of prolonged hypoxia and reoxygenation, amplification of EDHF-mediated relaxation induced by preconditioning is associated with an increase of TRPV4 expression in endothelial cells. Preconditioning also increases eNOS phosphorylation to provide cardioprotection through a TRPV4-dependent mechanism [87]. These findings laid the foundation for future development of endothelial TRP channel-targeting strategies for postischemic myocardial protection.

Cardioprotective potential of endothelial-specific miRNA

As a class of ~18-22 nucleotide noncoding RNAs, microRNAs (miRNAs) are important regulators of gene expression at the post-transcriptional level by inhibiting mRNA translation and/or promoting mRNA degradation. Research conducted in the past decade has revealed the significance of miRNAs in pathophysiological processes involved in ischemic heart disease. The differential expression of miRNAs in myocardial I/R injury and the factors that influence the miRNAs expression profile such as hypoxia-inducible factors (HIFs) and ROS have been recently reviewed in depth elsewhere [95, 96]. miRNAs expressed in endothelial cells are involved in the process of vascular inflammation and angiogenesis as well as in the regulation of vascular tone and endothelial cell barrier function [97]. For example, by regulating endo-

thelial expression of VCAM-1 and targeting sprouty-related protein and phosphoionsitol-3 kinase regulatory subunit 2 to regulate vascular endothelial growth factor (VEGF) pathway, the endothelial-specific miRNA, miR-126, may provide additional control of I/R-induced vascular inflammation and angiogenesis. As evidence regarding the roles of endothelial miRNAs in cardiovascular pathophysiology continuously grows, the potential of miRNAs therapeutics become more and more attractive. Currently, the therapeutic use of miRNAs is being attempted through two approaches: inhibition through antisense methodologies, and overexpression through mimicry either by viral vector-mediated gene expression or use of oligonucleotide miRNA. Despite rapid development in this field, much remains to be solved or improved. One example is the possible "promiscuous" effect of anti-miRNAs, which is because miRNAs can bind to targets with different sequences and sequences outside the seed region may be biologically active. Efforts shall be made to assess the on-target effect of antisense sequences on diseased tissues. In addition, it has to be aware that to date studies of miRNA have been largely confined to in vitro cell cultures and small animal experiments, therefore before miRNA therapeutics can be translated into the clinical setting, large animal studies are required.

Cardioprotective potential of cell-based therapy

It was reported that endothelial function in humans is associated with the number of circulating endothelial progenitor cells (EPCs) [98]. Increases in number of EPCs and NO production mediate the endothelial protection conferred by ischemic preconditioning in humans [99]. Intracoronary delivery of progenitor cells in patients with chronically occluded coronary arteries led to improvement in coronary flow reserve and cardiac function at 3-months post-transplant [100]. A recent study showed that the EPC-driven postischemic myocardial protection is partially mediated by activation of the VEGF-PI3K/Akt-eNOS pathway [101]. Microvesicles released from EPCs are thought to play a role in the endothelial protection conferred by EPCs. In vitro co-culture experiments with EPC-derived microvesicles and H/R-exposed human microvascular endothelial cells provided evidence for the role of microvesicles-carried RNAs (i.e., miR126) in control of ROS production and PI3K/eNOS/NO pathway in vascular endothelial cells [102]. MacArthur and colleagues recently developed a hydrogel delivery system enabling the sustained release of a bioactive EPC chemokine, which induces continuous homing of EPCs and effectively improves left ventricular function in a rat model of myocardial infarction [103]. However, one must admit that knowledge of progenitor cells still remains inadequate and more preclinical and clinical studies are needed.

Figure 2 summarizes the existing and potential endothelium-targeting strategies for postischemic myocardial protection.

In summary, the significance of vascular endothelial dysfunction in myocardial I/R injury makes endothelium an attractive therapeutic target for postischemic myocardial protection. Development of approaches controlling endothelial cell activation and more specific interventions targeting endothelial redox mechanisms will help alleviate myocardial injury following I/R. Endothelial progenitor cells represent an emerging cell-based strategy for promoting vascular repair and restoring microvascular perfusion of ischemic myocardium. Moreover, new insights into molecular mechanisms of endothelial dysfunction in relation to NO and EDHF during I/R and cardioplegic intervention, such as connexin proteins and ion channels, may lead to novel therapeutic strategies with the potential to improve prognosis of myocardial ischemia. The great hope of these endothelium targeting strategies for postischemic myocardial protection remains to be realized with further preclinical and clinical research.

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Disclosure of conflict of interest

None.

Abbreviations

Cx, Connexin; CYP450, Cytochrome P450 epoxygenases; DAMPs, Damage-associated molecule patterns; EDHF, Endothelium-derived hyperpolarizing factor; eNOS, Endothelial nitric oxide synthase; EPC, Endothelial progenitor cell; EETs, Epoxyeicosatrienoic acids; HSP27, Heat shock protein 27; HIFs, Hypoxia-inducible factors; H/R, Hypoxia/reoxygenation; ICAM, Intercellular adhesion molecule; IKCa, Intermediate conductance Ca2+-activated K+ channel; I/R, Ischemia/reperfusion; BKCa, Large-conductance Ca2+-activated K+ channel; MAPKs, Mitogen-activated protein kinases; MCP-1, Monocyte chemoattractant protein 1; NO, Nitric oxide; NF-κB, Nuclear factor kappa-B; ONOO-, Peroxynitrite; PECAM-1, Platelet endothelial cell adhesion molecule 1; ROS, Reactive oxygen species; she, Soluble epoxide hydrolase; SKCa, Small conductance Ca2+-activated K+ channel; SOD, Superoxide dismutase; BH4, Tetrahydrobiopterin; TLRs, Toll-like receptors; TRP channel, Transient receptor potential channel; VCAM-1, Vascular cell adhesion molecule-1; VEGF, Vascular endothelial growth factor.

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