Ischemic heart disease (IHD) is a significant cause of morbidity and mortality in Western society. Although interventions, such as thrombolysis and percutaneous coronary intervention (PCI), have proven efficacious in ischemia and reperfusion injury, the underlying pathological process of ischemic heart disease, laboratory studies suggest further protection is possible, and an expansive research effort is aimed at bringing new therapeutic options to the clinic. Mitochondrial dysfunction plays a key role in the pathogenesis of ischemia and reperfusion injury and cardiomyopathy. However, despite promising mitochondria-targeted drugs emerging from the laboratory, very few have successfully completed clinical trials. As such, the mitochondrion is a potential untapped target for new ischemic heart disease and cardiomyopathy therapies. Notably, there are a number of overlapping therapies for both these diseases, and as such novel therapeutic options for one condition may find use in the other. This review summarizes efforts to date in targeting mitochondria for ischemic heart disease and cardiomyopathy therapy and outlines emerging drug targets in this field. (Circ Res. 2012;111:1222-1236.)

Key Words: bioenergetics ■ clinical trial ■ ischemia ■ permeability transition pore ■ reperfusion ■ therapeutics

Mitochondrial Energetics in Normal Cardiac Function

The main function of cardiac mitochondria is ATP synthesis via oxidative phosphorylation (Ox-Phos). After
substrate oxidation, reducing equivalents generated by the tricarboxylic acid cycle are used by the respiratory chain (RC) to generate a transmembrane potential (mitochondrial membrane potential) that drives ATP synthesis. Under normal conditions, the adult heart relies mostly on fatty acids to fuel Ox-Phos, with 10% to 30% of total ATP derived from glucose.4 Substrate utilization does vary under normal conditions, with increased aerobic glycolysis found postprandially and during exercise, and in relation to substrate availability and circadian rhythm.5–8 In contrast, the immature heart relies predominately on glucose or lactate to provide carbon to the tricarboxylic acid cycle, with a transition to the mature fat-burning phenotype around 1 week of age.9

Mitochondrial Dysfunction in CM
The major cause of acute CM, the precursor to heart failure, is myocardial ischemia (see section Mitochondrial Dysfunction in IHD), whereas less acute forms of CM are idiopathic, inherited, or caused by chronic diseases, such as hypertension and diabetes mellitus. Regardless of the cause, altered mitochondrial bioenergetics appear to play a substantial role in CM.10

Under pathological conditions, such as CM, the normal metabolic flexibility of the heart is superseded by activation of a fetal metabolic program, with a preference for glucose over fat as the substrate for Ox-Phos.11 Although such changes lower the O2 consumed per ATP produced, the yield of ATP per substrate also decreases. Such inefficient metabolism lowers ATP and phosphocreatine levels and decreases metabolic reserve and flexibility.5,6,8 Interestingly, adult hearts that adopt this neonatal metabolism are refractory to protection by ischemic preconditioning (IPC, see section Current Mitochondrial Treatments for IHD) like the neonatal heart.13,14 However, IPC itself comprises metabolic remodeling, suggesting that altered metabolism may be an adaptive response to ischemia, becoming pathological if the stimulus persists.

Metabolic alterations are also an important component of inherited CMs.6,15 Hypertrophic CM (the most common form with a prevalence of ≈0.2% in the US population) is generally caused by contractile protein mutations that raise the energetic costs of contraction, leading to secondary energetic failure. However, ≈27% of hypertrophic CM cases are caused by inborn errors of metabolism, leading to primary energetic failure.16 In addition, ≈7% of dilated CM is caused by mutations in metabolism genes. Primary mitochondrial genetic disorders are also the most common causes in noncompaction CM,17 and 2 recent reports suggest restrictive CM and arrhythmogenic right ventricular dysplasia may be plagued by metabolic abnormalities.18,19

Mitochondrial Dysfunction in IHD
The primary pathological event in IHD is acute myocardial infarction (AMI) caused by coronary artery obstruction. Whereas permanent occlusion often results in cardiac remodeling and hypertrophy, transient occlusion is also detrimental to cardiac function, and the reperfusion phase of IR injury is particularly injurious to mitochondria.

A wide spectrum of mitochondrial derangements occurs in the post-IR heart, including the following: (1) Inhibition of respiratory complexes20 and the adenine nucleotide translocase,21 (2) Increased proton leak of the inner membrane,22 (3) Oxidation of the phospholipid cardiolipin and associated membrane protein dysfunction,23 (4) Excessive generation of reactive oxygen species (ROS),24 (5) Opening of the permeability transition (PT) pore, and release of cell death–inducing proteins, including cytochrome c,25 (6) Catastrophic nucleotide depletion,26 and (7) Mitochondrial Ca2+ overload.27 These phenomena are summarized in Figure 1, and while a brief understanding is helpful to understand the mechanisms of protection by mitochondrial therapeutics, in-depth discussions are available elsewhere.28

Current Mitochondrial Treatments for CM
Several studies have attempted to treat heart failure associated with CM by targeting bioenergetic dysfunction. The general strategy for primary mitochondrial genetic disorders is supplementation with cofactors, such as coenzyme Q, carnitine, riboflavin, and thiamine, or antioxidants such as ascorbate. The efficacy of such treatments for CM is unclear,16,17,29 because most mitochondrial disease trials have focused on neurological complications of these disorders rather than CM. Several groups have tested the coenzyme Q analog idebenone, and in some studies this molecule slowed or prevented progression of hypertrophic CM in patients with Friedreich’s ataxia (a disease of iron-sulfur cluster assembly that affects the RC), however a more recent trial found no improvement in left ventricle mass or function.29,30

Notably, even in CMs of unknown or nonmitochondrial causes, the mainstay clinical agents are coenzyme Q and carnitine,31 particularly if carnitine deficiency is evident on blood
testing. Idebenone has also been used for nonmitochondrial CM, and it improved cardiac function in a mouse model of duchenne muscular dystrophy. Whereas a subsequent phase II trial in duchenne muscular dystrophy patients failed to show significant improvement in cardiac function, a promising trend was seen and a larger trial is underway.

Another potential therapy for mitochondrial disorders is dichloroacetate, which stimulates pyruvate dehydrogenase to shunt pyruvate into Ox-Phos. Dichloroacetate has yielded mixed results in treating neurological symptoms of mitochondrial disorders, leading to suggestions that it may only be useful in patients with pyruvate dehydrogenase deficiency. The ability of dichloroacetate to treat mitochondrial CM has not been tested, but in patients with a mixture of non-ischemic and ischemic dilated CM, dichloroacetate showed mixed effects on myocardial O2 consumption and mechanical efficiency.

The amino acid l-arginine has also been proposed to treat mitochondrial disorders, and in patients with mitochondrial CM, arginine increased aerobic metabolism and myocardial efficiency. The mechanism appeared to be independent of myocardial blood flow (ie, arginine as a substrate for NO production), possibly occurring via supplying the tricarboxylic acid cycle with 2-oxyglutarate. Another drug of interest is perhexiline, which significantly increased phosphocreatine/ATP ratio, corrected diastolic dysfunction, and increased exercise capacity in CM patients, by inhibiting fatty acid oxidation (FAO) and increasing glucose utilization. Other potential metabolic modulators, as well as nonpharmacological therapies, such as exercise training, may prove efficacious in treating CM. Pharmacological options are discussed further in section Mitochondrial Metabolism.

Current Mitochondrial Treatments for IHD

Many therapies are aimed at slowing the progression of IHD, including statins to slow atherosclerotic lesion formation, antianginals to improve flow to transiently ischemic tissue, and antplatelet agents to impede clotting at plaque rupture. Whereas these agents do impact the incidence of ischemic events, there are limited therapies to protect myocardial function once an ischemic insult occurs.

In some situations, cardiac ischemia can be anticipated in advance, such as during cardiac surgery or balloon inflation during PCI. Careful quantification of cardiac damage using biomarkers can allow clinical trials to identify protective strategies. As such, in cardiac surgery, cardioplegia (controlled arrest of the heart, dropping myocardial energy requirements),
Table. Clinical Development of Mitochondrial Therapies for Cardiomyopathy and Ischemic Heart Disease

<table>
<thead>
<tr>
<th>Agent</th>
<th>Target/MOA</th>
<th>Clinical Development/Usage</th>
<th>Status of Cardiac Clinical Development</th>
<th>Clinical Trial</th>
<th>References</th>
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<td>PT pore inhibitors</td>
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<td>Edaravone</td>
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<td>In-use (stroke, Japan)</td>
<td>Phase IV (AMI)</td>
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<td>Nutriceutical</td>
<td>Cardioplegia additive</td>
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(continued)
hypothermia, \( \beta \)-blockers, and tight glycemic control are all currently used for cardioprotection.

Recently, protecting the heart with IPC has begun to translate into the clinic. In this method, short intermittent IR cycles are administered before index ischemia, yielding reduced infarct size and improved postischemic function. IPC has completed successful clinical trials in both PCI and cardiac surgery, however it is usually accomplished by repeatedly cross-clamping the aorta, which may increase the risk of cerebral emboli or aortic vascular damage. In this regard, it is notable that transient occlusion at sites remote from the heart (remote ischemic preconditioning) is also cardioprotective, with clinical studies showing remote ischemic preconditioning efficacy in cardiac and noncardiac surgery and PCI. However, failure of other remote ischemic preconditioning trials suggests protocol optimization may be required. As shown in Figure 2, the mechanism of IPC protection is incredibly complex, with mitochondria (and particularly mitochondrial \( \mathrm{K}^+ \) channels, see section Mitochondrial Potassium Channels) playing a key role.

Related to IPC, and more clinically tractable, is volatile anesthetic preconditioning (APC), in which halogenated anesthetics (iso-/des/-sevo-flurane) are known to activate many of the same intracellular signaling events as IPC. APC is also thought to open mitochondrial \( \mathrm{K}^+ \) channels (see section Mitochondrial Potassium Channels).

Unfortunately, the unanticipated nature of AMI makes it difficult to offer protection by preconditioning, and the current standard of care is early reperfusion via PCI.
or thrombolytics. Luckily it appears that intermittent reperfusion (ischemic postconditioning [IPoC]) can confer some of the protective benefits of IPC. Clinical trials have confirmed that IPoC during PCI can decrease infarct size and improve post-MI heart failure. Remote ischemic postconditioning has also shown success in clinical trials. The mechanism of IPoC is thought to involve opening of mitochondrial K⁺ channels and inhibition of the mitochondrial PT pore.

Targeting metabolism is another strategy to protect the heart in AMI. Simply reducing cardiac mitochondrial workload with β-blockers has proven effective in reducing mortality after AMI and coronary artery bypass graft (CABG) surgery, although how well this extends to noncardiac surgery patients with IHD is debated. Another metabolic therapy is adenosine, which reduces infarct size when delivered at reperfusion in animal models. Early adenosine clinical trials were successful, but later trials (AMISTAD) showed infarct reduction only in patient subsets, with a trend toward more adverse events and no improvement in hospital outcomes. A large trial (AMISTAD-II) in patients undergoing PCI reported a 60% drop in infarct size, but no change in outcomes at 6-month follow-up. Further trials using adenosine and analogs, such as AMP579, were disappointing, and meta-analysis revealed no change in overall outcomes.

Somewhat related to adenosine is acadesine (5-aminomidazole-4-carboxamide-1-β-D-ribofuranoside), an ATP metabolite that was originally thought to replenish post-IR adenine nucleotides. Although acadesine failed to elevate cellular ATP, it still elicited cardioprotection, leading to suggestions that its cardioprotective effects may result from activation of AMP-dependent protein kinase, an important regulator of metabolism (see section Mitochondrial Metabolism). Further study showed that acadesine increased extracellular adenosine levels and decreased myocardial stunning, leading to successful clinical trials in CABG and a large phase II trial (Acadesine1024), which yielded lower 2-year mortality in patients with perioperative myocardial infarction. However, a recent multicenter phase III trial (National Clinical Trials [NCT] 00872001) was terminated early (Merck Inc 2010 second quarter Securities and Exchange Commission filing) because of futility.

Another class of drugs that have largely failed to alleviate IR injury in the clinic are antioxidants. Early reports on the involvement of ROS in IR pathogenesis were shortly followed by animal studies demonstrating exogenous superoxide dismutase or catalase or both could elicit cardioprotection. However, these reagents failed in large animal and human trials. Consistent with xanthine oxidase as a source of ROS in IR injury, the xanthine oxidase inhibitor allopurinol was found to be cardioprotective in animal models, but also failed in clinical trials. Other small molecule antioxidants (including coenzyme Q, mercaptopropionyl glycine and others) have also been tested in IR injury, but all yielded mixed results in large animal or human studies. Despite this, antioxidants remain key ingredients in cardioplegia.

Overall, numerous drugs have shown promise in the laboratory for protecting the heart in IHD via mitochondrial mechanisms, but none has passed clinical trials. The reasons for these failures have been discussed extensively elsewhere, and may include unforeseen drug interactions, the inappropriate nature of animal models, confounding risk factors, such as diabetes mellitus and aging, or poor drug delivery to ischemic tissue. Furthermore, many cardioprotective drugs may actually block endogenous cardioprotective signals; for example, IPC requires ROS, such that antioxidants block IPC.

Emerging Mitochondrial Therapeutic Targets

Despite the wave of failed CM and IHD candidate therapeutics to date, the mitochondrial research community has made significant strides in deciphering mechanisms of cardioprotection (Figure 2), resulting in development of more specific and
targeted molecules. Some of these newer candidates are discussed below, categorized by the mitochondrial phenomena which they target.

Mitochondrial Permeability Transition Pore

Opening of the mitochondrial PT pore is a major driver of necrotic cell death in IR injury. The identity of the molecules that assemble to form the PT pore remains somewhat controversial, however transgenic animal models have demonstrated pore regulation by the adenine nucleotide translocase, the phosphate carrier, and cyclophilin D (CypD), with the role of the voltage-dependent anion channel less clear. Other proteins implicated in the PT pore or its regulation include hexokinase II, which links the pore to cellular metabolism, and mitochondrial translocator protein, which interacts with voltage-dependent anion channel. Drugs targeting these PT pore components are candidates for cardioprotective therapeutics.

The first drug shown to inhibit PT pore opening was cyclosporine A (CsA), which binds to CypD. CsA protects against IR injury in myocytes and intact hearts, and a pilot clinical trial demonstrated 20% infarct reduction with CsA administration before PCI in myocardial infarction patients, with benefits still evident 6 months later. A phase III trial (NCT01502774) is currently underway. Notably, CsA is also used for immunosuppression after organ transplant, mediated by its binding to calcineurin. Calcineurin is also a well-known hypertrophic signaling molecule, and hypertrophy attributed to CsA usage has been reported in transplant patients. Thus, CypD-specific CsA analogs have been developed, including sanglifehrin A, and Debio025 (Debio025 [NCT00537407 and NCT00924118, NCT01098409]). These molecules are cardioprotective in animal models of IR injury, although none has yet progressed to clinical trials. A mitochondria-targeted CsA has also been developed, exhibiting higher potency and improved CypD affinity. The translocator protein ligands SSR180575 and TRO40303 were also shown to reduce IR injury in various cell and animal models, and TRO40303 is entering clinical trials (NCT01374321).

NO-Based Mitochondrial Therapies

The role of NO in both endogenous cardioprotection and in mediating protective effects of drugs has been reviewed extensively elsewhere. In addition to classic NO signaling via cGMP/cGMP-dependent protein kinase, which feeds into reperfusion injury salvage kinase (RISK) signaling (see section RISK Pathway), nonclassic functions of NO are also implicated in cardioprotection, including protein S-nitrosation and the generation of nitro-lipids. Mitochondria are a critical site of action for these signals, in particular the S-nitrosation of respiratory complex I resulting in reversible inhibition. Furthermore, CypD has been identified as a target of S-nitrosation leading to attenuation of PT pore opening.

A series of molecules have been developed to deliver NO to mitochondria. The first such agent was S-nitroso mercaptopropionyl glycine, derived from the antioxidant mercaptopropionyl glycine-mercaptopropionyl glycine. S-nitroso mercaptopropionyl glycine was actively sequestered into mitochondria and exhibited cardioprotection in animal models of IR injury. Protection was associated with complex I S-nitrosation and was lost in a complex I mutant mouse. A more recent development is MitoSNO1, an NO donor based on the triphenylphosphonium (TPP) mitochondrial targeting moiety, which exhibits cardioprotection when delivered at reperfusion. A related molecule is 2-hydroxylamine-vinyl-TPP, which requires metabolism by intramitochondrial peroxidases to release NO, although its efficacy in IR injury is not yet known.

Another set of NO-derived molecules that target mitochondria are the nitro-lipids. These molecules are generated endogenously inside mitochondria during IPC, and when added exogenously they protect in various models of IR injury. Such protection occurred on a timescale too fast for gene regulatory effects usually attributed to nitro-lipids (e.g., peroxisome proliferator activated receptor isoform γ, nuclear factor κB, nuclear factor erythroid 2-related factor 2/Kelch-like ECH-associated protein 1). Instead, nitro-lipid protection occurred via covalent modification of adenine nucleotide translocase, eliciting a mild increase in mitochondrial uncoupling, which is known to confer cardioprotection. Nitro-lipid derivatives are currently in early stage clinical development.

Finally, significant attention has recently focused on the anti-ischemic properties of nitrite (NO−). Several studies have implicated mitochondria, and in particular complex I, as a downstream target of NO− in cardioprotection. Injectable sodium NO− is federal drug administration approved as a cyanide poisoning antidote, and several NO− clinical trials are underway for cardiac ischemia (NCT01401517, NCT00924118, NCT01098409).

Mitochondrial Potassium Channels

The mitochondrial inner membrane contains numerous ion channels, and many studies have suggested a role for mitochondrial K+ channels in cardioprotection. The mechanism of such protection is unclear, and may involve mild uncoupling, diminishing the mitochondrial membrane potential driving force for Ca2+ overload and ROS generation (see section Mitochondrial Dysfunction in Ischemic Heart Disease). Mild swelling associated with mitochondrial K+ influx may also regulate the PT pore.

A mitochondrial KATP channel (mKATP) is reported to play a critical role in cardioprotection by IPC. One of the most widely tested mKATP openers is diazoxide, which has demonstrated protection from IR injury in multiple cell and animal models. Two small clinical trials in CABG patients reported cardioprotection in patients pretreated with diazoxide or undergoing surgery supported by diazoxide-supplemented cardioplasia. Other mKATP openers, including BMS-191095, pinacidil, cromakalim, minoxidil, and nicorandil (Nicorandil failed in phase III clinical trials of acute MI [J-WIND]), are cardioprotective in animal models.

Although the molecular identity of mKATP is unclear, a link exists between respiratory complex II and mKATP, such that mKATP openers inhibit complex II, and complex II inhibitors open mKATP. It is hoped that definitive identification of this channel will aid in the design of mKATP-specific drugs. Notably, many pharmaceuticals, including fluoxetine, and...
some sulfonylureas inhibit mKATP abrogating cardioprotection by IPC.\textsuperscript{140,141}

In addition to mKATP, mitochondria are also thought to contain a large conductance K$^+$ channel (BK) encoded by the SLO gene family,\textsuperscript{142} which is implicated in cardioprotection by APC.\textsuperscript{143} Until recently, consensus held that the mitochondrial BK channel was the SLO1 isoform, based on cardioprotection by the SLO1 opener NS1619 in animal models of IR injury.\textsuperscript{144} However, NS1619 acts on several ion channels\textsuperscript{145,146} and other mitochondrial targets.\textsuperscript{147} A more specific SLO1 activator (NS11021) is also cardioprotective,\textsuperscript{148} but controversy remains because it was recently shown in Caenorhabditis elegans and mice that SLO1 is dispensable for APC, and SLO2 is necessary for APC in the worm.\textsuperscript{139} There are currently no SLO2 specific activators, but SLO2 may be a future therapeutic target.

**Mitochondrial Antioxidants**

As discussed in section Current Mitochondrial Treatments for Ischemic Heart Disease, multiple generic nontargeted antioxidants have been clinically unsuccessful in preventing IR injury, although several newer antioxidants are in clinical trials, including melatonin (NCT01172171), mangafodipir (NCT00966563), and edaravone (NCT00265239). In addition, a potentially novel therapeutic approach is the targeting of antioxidants to mitochondria.

A key chemical strategy for mitochondrial targeting is the TPP$^+$ moiety (see section NO-Based Mitochondrial Therapies). First exploited in the 1970s as a method to measure mitochondrial membrane potential,\textsuperscript{148} several TPP$^+$-conjugated antioxidants were developed in the 1990s. The most prominent of these, mitochondrially targeted coenzyme Q,\textsuperscript{149} was cardioprotective in a rat model of IR injury,\textsuperscript{149} and is being developed clinically for other indications (NCT00433108). Other antioxidants conjugated to TPP$^+$ include $\alpha$-tocopherol (MitoE) and nitroxides.\textsuperscript{150} As discussed in section NO-Based Mitochondrial Therapies, a TPP$^+$-conjugated NO$^+$ donor confers potent cardioprotection.

Somewhat related to TPP$^+$ conjugates are the Szeto Schiller peptides, which contain positively charged amino acids and a dimethyl tyrosine residue. Originally developed as opioid peptide analgesics, after the discovery they were cardioprotective, their mechanism was assigned to a mitochondrial antioxidant activity.\textsuperscript{152} The lead compound in this series, Szeto Schiller-31 (N-Arg-dimethyl tyrosine-Asn-Phe-NH$_2$), has been renamed Bendavia and is in clinical trials for AMI (NCT01572909).

Bioactivation is another strategy to target drugs to mitochondria, an example being the $\omega$-(1-methyl-1H-imidazol-2-ylthio) alkanoic acids. These inactive prodrugs are metabolized by fatty acid $\beta$-oxidation in the mitochondrial matrix to reveal a methimazole antioxidant.\textsuperscript{153} $\omega$-(1-methyl-1H-imidazol-2-ylthio) alkanoic acids were shown to protect isolated cardiomyocytes from IR injury, in a manner inhibited by the $\beta$-oxidation blocker etomoxir. Such molecules may be particularly efficacious in the heart, given its preference for fatty acids as a metabolic substrate (see section Introduction).

**RC Inhibitors**

Ischemia inhibits Ox-Phos because of lack of O$_2$ as the terminal electron acceptor for the RC. Thus, it is somewhat paradoxical that many chemical RC inhibitors are protective in ischemia. This includes inhibitors of complex I (rotenone,\textsuperscript{154} amobarbital,\textsuperscript{155} S-nitrosothiols, NO$^+_2$), complex II (diazoxide,\textsuperscript{131} atenol A5\textsuperscript{146}), complex III (antimycin A156), and complex IV (CO,\textsuperscript{157} H$_2$S,\textsuperscript{158} NO$^+_2$). The mechanism of protection may involve inhibition of the large burst of ROS generation and Ca$^{2+}$ overload that occurs at reperfusion, with inhibitor washout allowing a more gradual reintroduction of electron flux to the RC, somewhat akin to IPC or slow reperfusion.\textsuperscript{159} Although neurological side effects associated with RC inhibitors (eg, Parkinson disease for rotenone, Huntington disease for 3-nitropropionic acid) may preclude their clinical applicability, amobarbital is clinically available and is protective when delivered at reperfusion.\textsuperscript{155}

Related to RC inhibitors, mild uncoupling of Ox-Phos by chemicals such as carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone can also elicit cardioprotection. The mechanism underlying this is unclear but may include inhibition of ROS generation and Ca$^{2+}$ overload.\textsuperscript{124,160} As discussed in section NO-Based Mitochondrial Therapies, cardioprotection by nitro-lipids may also proceed via mild uncoupling.

**RISK Pathway**

In IR injury, the PT pore remains closed during ischemia and opens early in reperfusion (Figure 1).\textsuperscript{125} Opening of the pore at reperfusion is regulated by the RISK signaling pathway.\textsuperscript{167} This pathway is implicated in IPC and IPoC\textsuperscript{168} and involves numerous kinases that converge on phosphorylation and inhibition of glycogen synthase kinase-3$\beta$,\textsuperscript{161} which is thought to phosphorylate PT pore components (Figure 2).\textsuperscript{163} The glycogen synthase kinase-3$\beta$ inhibitors SB-216763 and lithium improve post-IR cardiac function in animal models\textsuperscript{164} (although mK$_{ATP}$ channels may also be involved\textsuperscript{165}).

Many extracellular signals (eg, insulin$^{166}$) can activate RISK signaling via receptors, and NO$^+$ is also implicated in RISK signaling.\textsuperscript{167,168} Several drugs also exert cardioprotective effects by activating RISK, most notably statins (simvastatin,\textsuperscript{169} mevastatin,\textsuperscript{170} atorvastatin\textsuperscript{171}) via a mechanism not linked to cholesterol lowering. Multiple clinical trials (ARYMDA, NAPLES) and a meta-analysis\textsuperscript{172} have demonstrated cardioprotection with statin administration before emergent PCI. Further clinical trials are ongoing to deliver statins at reperfusion for patients presenting with myocardial infarction (NCT01050348, NCT01334671, NCT00772564). Many other drugs that activate RISK signaling are reviewed elsewhere.\textsuperscript{173,174}

**Aldehyde Dehydrogenase 2**

A relatively new target for mitochondrial protection in IR injury is aldehyde dehydrogenase isoform 2 (ALDH2), a mitochondrial enzyme which removes toxic aldehydes, such as 4-hydroxy-2-nonenal. 4-hydroxy-2-nonenal has been implicated in cardiac IR injury, and can inhibit metabolic enzymes and RC complexes, and open the PT pore.\textsuperscript{175}

Overexpression of ALDH2 decreases 4-hydroxy-2-nonenal levels, yielding decreased infarct size and increased...
Mitochondrial Metabolism

One of the earliest methods to improve mitochondrial metabolism in IR was the use of glucose-insulin-potassium (GIK) supplementation. In addition to GIK, insulin alone can drive both glucose and fatty acids into the cell to support metabolism.180 GIK has shown mixed results in CABG surgery, with a recent meta-analysis showing no improvement in the incidence of arrhythmia or mortality.182 However, with recent emphasis on tight glucose control during cardiac surgery, many patients receive intraoperative GIK anyway.183 Results in AMI have been consistently negative, although a recent trial (NCT0091507) of GIK delivery by emergency personnel in the prehospital setting yielded a 50% drop in in-hospital cardiac arrest and mortality.184

FAO is also an attractive drug target in ischemia, because it uses more O2 per ATP produced (versus glucose). During IR injury, catecholamine-induced increases in serum fatty acid levels may enhance FAO,185 and indeed reperfusion with fatty acid free serum is cardioprotective.186 Inhibitors of carnitine palmitoyltransferase 1 (which transports fatty acids into mitochondria), such as etomoxir, have also demonstrated cardioprotection in animal models of IR injury,177 and improved hemodynamics in clinical trials for heart failure.188 Another carnitine palmitoyltransferase 1 inhibitor, perhexiline, corrected diastolic dysfunction, and increased exercise capacity in patients with hypertrophic CM.189 Oxifenicine shows similar effects but is not used in humans.189

Other drugs that inhibit FAO are the antianginals trimetazidine and ranolazine.190 A meta-analysis of trimetazidine in heart failure demonstrated improved ventricular function and decreased mortality, cardiovascular events, and hospitalizations.191 In IR injury it exhibited mixed results in both animal models192,193 and clinical trials, demonstrating protection when administered before CABG,194 but no protection in AMI except for a cohort of patients who did not receive thrombolysis.195 Ranolazine is reported to shift cell metabolism from FAO toward glycolysis,196 although it may also have effects on cellular Na+ and Ca2+ homeostasis197 or protection of complex I.198 In animal models of heart failure ranolazine reduced diastolic dysfunction and improved left ventricle efficiency,199 and in IR injury it decreased both infarct size and cardiac enzyme release and improved postischemic ventricular function.200 Clinical trials confirmed its efficacy in treating angina but showed no protection in AMI.201 Further ranolazine trials are in development for PCI (NCT01491061). Despite the consensus that fatty acids are toxic to the ischemic heart, it is notable that carnitine supplementation is paradoxically cardioprotective in animal models of IR injury.202 In clinical trials, carnitine prevented left ventricular remodeling after ST segment elevation myocardial infarction (CEDIM-I) and reduced 5-day mortality, however this mortality benefit was nonsignificant at 6 months (CEDIM-II).203,204

Global regulators of metabolism may also be therapeutic targets in IHD. An example is AMP-dependent protein kinase, which upregulates both FAO and glucose utilization in response to decreased ATP levels. Whether increased AMP-dependent protein kinase is helpful or harmful to myocardium after IR injury is still unclear.205 The AMP-dependent protein kinase specific activator A-769662 is cardioprotective during IR injury,206 but activators such as acadesine or metformin may have nonspecific effects. Another important metabolic regulator is silent information regulator 2P homolog 1 (SIRT1), which is required for cardioprotection by acute IPC.207 Despite controversy regarding the SIRT1 activator resveratrol,208 SIRT1 activators are in clinical development (NCT00933530).

Similarly, SIRT3, which is located in the mitochondria, has also emerged as an important metabolic regulator known to deacetylate numerous mitochondrial proteins involved in FAO and Ox-Phos.209 Notably a mitochondrial acetyltransferase (GCN5L1) that is counterpart to SIRT3 has recently been described, although from a pharmacological standpoint, specific drugs that target mitochondrial SIRT3 (versus other SIRTs) have not yet been developed.210 Finally, it has recently emerged that mitophagy, the process by which damaged mitochondria are removed and recycled, is an important player not only in metabolic homeostasis, but is also critical for IPC.211,212

Conclusions and Future Directions

In summary, despite numerous failures in clinical development of drugs to treat IHD and CM, the pipelines of several companies still contain IHD and CM drugs, and many of these therapies appear targeted at mitochondria. Coupled with novel compounds which have not yet reached clinical trials, these are exciting times to be involved in drug development for these debilitating conditions. However, it should be emphasized that, despite the large number of mitochondrial drugs discussed in section Emerging Mitochondrial Therapeutic Targets, there is no guarantee that these molecules will be more efficacious in the clinic than those tested to date. Many of the same reasons that have been invoked to explain previous clinical failures also apply to these therapeutic candidates.87

In addition to the novel compounds discussed, other significant developments also deserve brief mention. The first is National Institutes of Health consortium for preclinical Assessment of Cardioprotective therapies (CAESAR), an National Institutes of Health-sponsored consortium which aims to bridge the gap between preclinical and clinical trials for promising cardioprotective therapies.213 By adopting standardized IR protocols in several animal models, and using practices common to clinical trials (double blinding, multiple centers, placebo controls), the consortium aims to put only the most promising therapies forward into human clinical trials. This will clearly be a valuable tool for researchers in the field.
Secondly, with a few exceptions, high-throughput screening has been underused as a drug development approach for IHD and CM. This may be because clinically relevant models of IR injury often use animals and are time consuming and expensive (ie, not amenable to high-throughput screening), whereas high-throughput screening-ready IR models may not be physiologically relevant. In a recent screen for molecules that alter cell metabolism, it was hypothesized that hits that promote glycolysis would be beneficial during ischemia, and indeed one such molecule (meclizine) was protective in heart failure. 

In another recent screen, we developed an IR injury model using a 24-well metabolic phenotyping device, with post-IR cell death and metabolism as end points. One hit from the screen was cloxynquin, which is closely related to the mitophagy-inducing drug cliquinol, currently in clinical trials for cancer (NCT00963495). Another was ipiriflavone, which was first proposed as an antiischemic drug 30 years ago. Further phenotypic screens of this type may yield novel cardioprotective drugs which engage the mitochondrial targets discussed herein (Table).

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Disclosures

None.

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