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JACC REVIEW TOPIC OF THE WEEK

Multitarget Strategies to Reduce Myocardial Ischemia/Reperfusion Injury

JACC Review Topic of the Week

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ABSTRACT

Many treatments have been identified that confer robust cardioprotection in experimental animal models of acute ischemia and reperfusion injury. However, translation of these cardioprotective therapies into the clinical setting of acute myocardial infarction (AMI) for patient benefit has been disappointing. One important reason might be that AMI is multifactorial, causing cardiomyocyte death via multiple mechanisms, as well as affecting other cell types, including platelets, fibroblasts, endothelial and smooth muscle cells, and immune cells. Many cardioprotective strategies act through common end-effectors and may be suboptimal in patients with comorbidities. In this regard, emerging data suggest that optimal cardioprotection may require the combination of additive or synergistic multitarget therapies. This review will present an overview of the state of cardioprotection today and provide a roadmap for how we might progress towards successful clinical use of cardioprotective therapies following AMI, focusing on the rational combination of judiciously selected, multitarget therapies. This paper emerged as part of the discussions of the European Union (EU)-CARDIOPROTECTION Cooperation in Science and Technology (COST) Action, CA16225. (J Am Coll Cardiol 2019;73:89-99)
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ABBREVIATIONS AND ACRONYMS

AMI = acute myocardial infarction

GIK = glucose/insulin/potassium

IPC = ischemic pre-conditioning

IPost = ischemic post-conditioning

IRI = ischemia-reperfusion injury

MI = myocardial infarction

MPTP = mitochondrial permeability transition pore

MVO = microvascular obstruction

NAC = *N*-acetylcysteine

NOS = nitric oxide synthase

PKG = protein kinase G

PPCI = primary percutaneous coronary interventions

RIC = remote ischemic conditioning

RISK = reperfusion injury salvage kinase

SAFE = survivor activating factor enhancement

STEMI = ST-segment elevation myocardial infarction

CARDIOPROTECTION: WHERE WE ARE TODAY

Despite success in animal studies, translation of cardioprotection to clinical practice has proven difficult (1,2). Several pharmacological treatments have failed, and although ischemic conditioning strategies are promising, effects are weak and, in some cases, inconsistent (3). Differences between preclinical models of transient myocardial ischemia and the clinical scenario in patients, including age, comorbidities, and cotreatments (4,5), may help to explain the difficulties in translation in some cases. In others, insufficient preclinical data or incorrect study design may be responsible (1-3). However, the notion is emerging from experimental studies that an important reason for the weak, inconsistent results obtained in patients may be the presence of multiple, partially redundant mechanisms of cell death during ischemia-reperfusion whose relative importance may depend on the conditions. According to the hypothesis we raise herein, targeting 1 mechanism at a time may be insufficient to produce a strong and robust effect in clinical situations where many uncontrolled variables usually coexist.

DIFFERENT TYPES OF CARDIOPROTECTIVE STRATEGIES

Over the past 3 decades, many cardioprotective strategies against myocardial ischemia-reperfusion injury (IRI) have been proposed (6). These can be broadly divided into several categories based on the protective modality, time of application, cellular target, and intracellular target (**Central Illustration**). The cardioprotective modalities that have been the most studied are based on either the controlled application of episodes of brief ischemia and reperfusion (ischemic conditioning), the administration of chemical substances (pharmacological), or the application of physical measures, such as hypothermia or electrical nerve stimulation.

Strategies based on ischemic conditioning include local pre-conditioning (IPC) and post-conditioning (IPost), and remote ischemic conditioning (RIC). The mechanisms of ischemic conditioning are incompletely understood but are probably multiple. IPC delays recovery of pH_i, prevents uncoupling of nitric oxide synthases (NOS) and subsequent generation of reactive oxygen and nitrogen species, and increases protein kinase G (PKG), reperfusion injury salvage kinase (RISK) and survivor activating factor enhancement (SAFE) signaling in reperfused cardiomyocytes (7). RIC appears to share with IPC an effect on nitrotyrosylation and preservation of PKG (8), but also acts on mitochondrial function and activates the RISK and SAFE pathways (9,10).

Cardioprotective strategies can also be classified according to the time they are applied, that is, before, during, or after ischemia. Here, we limit our discussion to treatments applied after the onset of ischemia, because when patients with ST-segment elevation myocardial infarction (STEMI) present, their heart is already ischemic. However, studies suggest that some cardioprotective agents or interventions (e.g., cariporide [11], hypothermia [12], metoprolol [13], glucose/insulin/potassium [GIK] [14], RIC [15]) may reduce myocardial infarction (MI) size when administered during the acute ischemic phase. In fact, RIC and metoprolol may protect the heart from ongoing ischemic injury (16,17), providing an opportunity to deliver the cardioprotective agent or intervention to the acute myocardial infarction (AMI) patient in the ambulance on the way to the cardiac catheterization laboratory. However, for STEMI patients undergoing primary percutaneous coronary interventions (PPCI), administering the cardioprotective therapy before reperfusion by PPCI can be challenging, because it never should delay the onset of reperfusion.

Treatments that protect from reperfusion injury should generally be applied as early as possible during reperfusion because most cell death occurs during the first minutes of reflow. An example of this is IPost (18). In the case of drugs, it is generally

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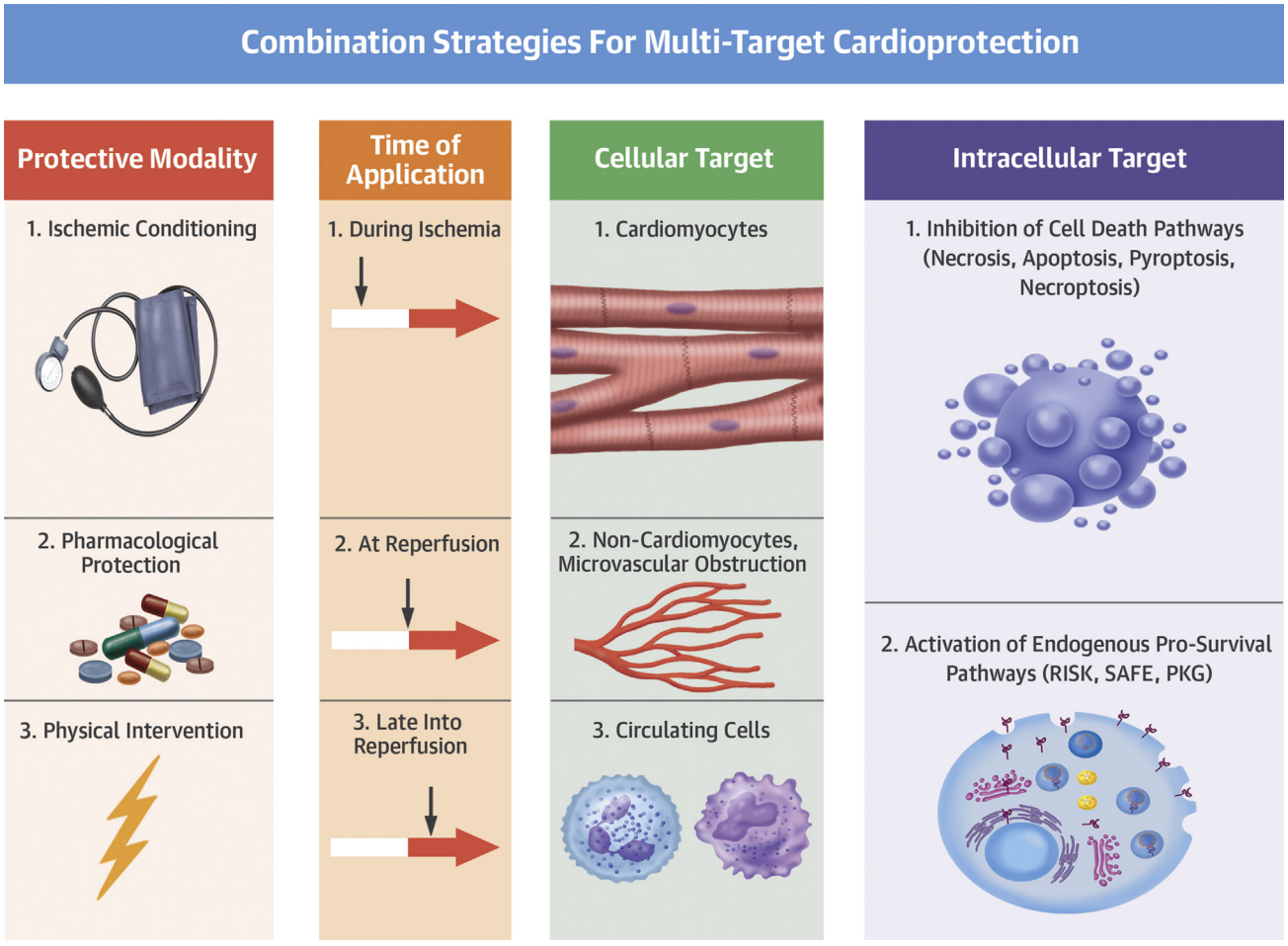
CENTRAL ILLUSTRATION **Multitarget Cardioprotective Strategies to Reduce Myocardial Infarction**



Davidson, S.M. et al. J Am Coll Cardiol. 2019;73(1):89–99.

Cardioprotective strategies fall into 4 broad categories, which may be combined in different manners to achieve multitarget cardioprotection. PKG = protein kinase G; RISK = reperfusion injury salvage kinase; SAFE = survivor activating factor enhancement.

preferable to administer them as early as possible during ischemia to ensure adequate myocardial concentration at the onset of reperfusion. Prior studies have shown that delaying the administration of some cardioprotective therapies (e.g., sanglifehrin-A [19], IPost [20]) until after reperfusion had already taken place failed to reduce MI size. On the other hand, there are limited experimental data suggesting that some cardioprotective agents or interventions (e.g., an inhibitor of phosphoinositide 3-kinase γ/δ (PI3Kγ/δ) [21], antiapoptotic agents [22], or delayed RIC [23]) may reduce MI size even when administered after the onset of reperfusion (20 min to 3 h into reperfusion), providing an opportunity to deliver the cardioprotective agent after PPCI when the STEMI patient is on the ward. However, this approach is based on the premise that MI size increases with reperfusion time, which remains controversial (4).

An additional consideration in the case of STEMI patients is that if drugs protecting against ischemic injury are given after coronary occlusion, they may fail to reach severely ischemic myocardium with little or no collateral flow (24,25). However, this situation may be rapidly changing due to the increasingly widespread use of potent antiplatelet drugs in these patients upon first medical contact (26), resulting in a growing proportion of infarcts being partially reperfused before PPCI. Physical therapies, such as hypothermia, may also reach ischemic myocardium when applied before reperfusion.

TABLE 1 Experimental Studies Illustrating the Potential for Additive Cardioprotection With Multiple Cardioprotective Agents or Interventions Having Distinct Targets Within the Cardiomyocyte

First Author, Year (Ref. #)	Experimental AMI Model	Cardioprotective Agents or Interventions	Cardioprotective Effect	Signaling Pathways
Schwiebert et al., 2010 (58)	In vivo rat	Xenon (20%) at reperfusion Hypothermia (34°C) at reperfusion	Additive effects on reducing MI size	Not investigated
Alburquerque-Béjar et al., 2015 (8)	In vivo pig	RIPerC GIK or exenatide at reperfusion	Additive effects of RIPerC with either insulin or exenatide	RIPerC—less oxidative stress and reduced eNOS uncoupling GIK and exenatide—shift to glycolysis
Sun et al., 2016 (59)	In vivo mice	NaHS (H_2S donor) at reperfusion SNAP (NO donor) at reperfusion	Additive effects on reducing MI size	NaHS—S-sulfhydration SNAP—S-nitrosylation

Experimental studies illustrating the potential for additive cardioprotective effects with 2 or more agents or interventions given acutely in combination during ischemia or shortly after reperfusion. Only those agents given in the short term in combination during ischemia or shortly after reperfusion, and demonstrating a reduction in infarct size that is additive are shown.

AMI = acute myocardial infarction; eNOS = endothelial nitric oxide synthase; GIK = glucose/insulin/potassium; MI = myocardial infarction; NaHS = Sodium hydrosulfide; NO = nitric oxide; RIPerC = remote limb ischemic per-conditioning during cardiac ischemia; SNAP = *S*-nitroso-*N*-acetylpenicillamine.

Cardioprotective strategies may be further categorized by their end targets (**Central Illustration**). The first group includes molecular targets involved in mainly necrotic cell death, such as ion exchangers and channels, proteases, reactive oxygen species, contractile elements, or constituents of the mitochondrial permeability transition pore (MPTP). These strategies have generally been based on the use of pre-existing pharmacological tools and have rarely progressed to clinical trials. One exception is cyclosporine A, which targets the MPTP. However, cyclosporine A produced inconsistent preclinical results and failed in clinical trials. Other forms of cell death may occur during acute myocardial IRI, including apoptosis, autophagy, necroptosis, and pyroptosis, all of which may contribute in varying degrees to final MI size following acute IRI and provide new targets for cardioprotection. A second group of targets includes activation of endogenous cardioprotective signaling pathways, including the NO/cGMP/PKG cascade, RISK and SAFE pathways, mitochondrial morphology, and cardiomyocyte metabolism. Inflammation contributes to post-MI injury and forms an additional target for its reduction (21,27). Translation of these individual targets to patients has met with variable success (3,6), but they could form part of a multitarget strategy.

Finally, cardioprotective strategies may be aimed at either protecting cardiomyocytes or non-cardiomyocyte cells, such as platelets or leukocytes (6). Although cardiomyocytes are the working cells in the heart and the most susceptible to IRI, the myocardium also contains a large number of other cell types that are important players in myocardial IRI, including endothelial cells, fibroblasts, smooth muscle cells, and neuronal cells. Some factors released by the endothelium and fibroblasts (i.e., the "secretome") such as microRNA (miRNA) and exosomes,

TABLE 2 Experimental Studies Illustrating the Potential for Additive Cardioprotection With Multiple Cardioprotective Agents or Interventions Combining Cardiomyocyte Targets With Noncardiomyocyte Targets*

First Author, Year (Ref. #)	Experimental AMI Model	Cardioprotective Agents or Interventions	Cardioprotective Effect	Cardiomyocyte and Noncardiomyocyte Targets
Koshinuma et al., 2014 (42)	Isolated guinea pig heart	Z-VAD during ischemia and first 30 min of reperfusion Necrostatin-1 during ischemia and first 30 min of reperfusion	Additive effects on reducing MI size	Z-VAD—apoptosis inhibition Necrostatin-1—necroptosis inhibition
Yang et al., 2015 (60)	In vivo rat	Cangrelor at reperfusion Endonuclease III at reperfusion	Additive effects of cangrelor and endonuclease III on reducing MI size	Cangrelor, $P2Y_{12}$ inhibitor—platelets Endonuclease III, targets mitochondrial DNA—cardiomyocyte
Alexopoulos et al., 2017 (61)	In vivo rabbit	Exenatide at reperfusion Cyclosporine-A at reperfusion parstatin 1-26 at reperfusion	Additive effects with exenatide combined with either cyclosporine-A or parstatin 1-26 on reducing MI size	Exenatide-GLP-1 signaling—cardiomyocytes Cyclosporine-A—MPTP cardiomyocytes Parstatin 1-26—inflammation
Audia et al., 2018 (41)	In vivo rat	VX-765 and ticagrelor or cangrelor at reperfusion	Additive reduction in MI size after 2-h and 3-day reperfusion.	$P2Y_{12}$ inhibitor—platelets VX-765, caspase 1 inhibitor—inhibition of cardiomyocyte pyroptosis

*Other criteria are as per **Table 1**.

MPTP = mitochondrial permeability transition pore; VAD = val-ala-asp; Z-VAD = Z-val-ala-asp. Other abbreviations as in **Table 1**.

TABLE 3 Experimental Studies Illustrating the Potential for Additive Cardioprotection With Multiple Cardioprotective Agents or Interventions Targeting Distinct Time-Points During Ischemia and Reperfusion*

First Author, Year (Ref. #)	Experimental AMI Model	Cardioprotective Agents or Interventions	Cardioprotective Effect	Timing of Intervention
Xin et al., 2010 (62)	In vivo rat	Limb RIPerC (4 × 5/5-min cycles) IPost (6 × 10/10-s cycles)	Additive effects on reducing MI size, reducing ROS at reperfusion and inhibiting apoptosis	Ischemia—limb RIPerC Reperfusion—IPost Additive effects on Akt and Erk1/2 phosphorylation
Yang et al., 2013 (44)	In vivo rat	Cangrelor at reperfusion Hypothermia (32°C–33°C) during ischemia Cariporide during ischemia	Additive effects of cangrelor combined with either cariporide or hypothermia on reducing MI size. Additive protection with all 3	Reperfusion—cangrelor, $P2Y_{12}$ inhibitor Ischemia—hypothermia, reduces energy consumption Ischemia—cariporide, Na^+/H^+ exchanger inhibitor

*Other criteria are as per Table 1.
IPost = post-conditioning; ROS = reactive oxygen species; other abbreviations as in Table 1.

may contribute to cardioprotective signaling (28,29). IRI may cause the death of noncardiomyocytes by various pathways including apoptosis (30). Furthermore, IRI may disrupt the endothelial barrier favoring myocardial edema (31,32), and may activate endothelial cells causing them to interact with circulating blood cells that may plug the microvessels, release molecules that affect cardiomyocyte function and tolerance to IRI, and infiltrate the myocardium. Platelets are some of the first hematopoietic cells to respond to IRI. Although activated platelets release factors that may exert cardioprotective effects during ischemia (33) strong evidence indicates that they may exacerbate reperfusion injury by mechanisms not dependent on vessel obstruction (34,35). They also form coaggregates with white blood cells (mainly neutrophils), and these plugs are distally embolized upon reperfusion contributing to microvascular obstruction (MVO) (27). MVO can also be caused by embolization from the recanalized coronary plaque and extrinsic compression secondary to edema formation upon reperfusion (36). Furthermore, areas of no-reflow and intramyocardial hemorrhage may develop due to extreme myocardial devastation (37). However they arise, MVO and no-reflow have the potential to cause further cardiomyocyte necrosis (38) and are clearly associated with adverse prognosis in patients with AMI (32).

MULTITARGET STRATEGIES FOR CARDIOPROTECTION

We define "multitargeted cardioprotective therapy" as additive or synergistic cardioprotective effects of multiple cardioprotective agents or interventions directed to distinct targets. There are also some specific examples where a single agent is known to have effects on multiple distinct targets and can therefore also be considered as a multitargeted strategy. The combination of agents or interventions to achieve multitarget cardioprotection may be broadly classified into 5 categories, although these are not mutually exclusive (**Central Illustration, Tables 1 to 5**). Each of these categories are discussed in the following sections using examples taken from animal experiments. Their applicability to patients is discussed in the following text.

MULTIPLE CARDIOPROTECTIVE AGENTS OR INTERVENTIONS WITH DISTINCT TARGETS WITHIN THE CARDIOMYOCYTE. Conceptually, the simplest approach to multitarget cardioprotection is to combine 2 or more agents or interventions, each of which has a distinct target within the cardiomyocyte. In this approach, it is important that each cardioprotective agent or intervention is at maximal "dose" (i.e., not subthreshold), and that the combination of agents or interventions confers additive benefit in terms of infarct size reduction. The intracellular targets can include prosurvival signaling pathways (e.g., the RISK, SAFE, and NO-cGMP-PKG cascades), cell death pathways (e.g., necrosis, apoptosis, autophagy, necroptosis, and pyroptosis), and cellular organelles (e.g., mitochondria, sarcoplasmic reticulum) (**Figure 1**). As such, maximal cardioprotection may require activation of complementary prosurvival pathways and/or inhibition of deleterious cell death pathways, as recently proposed in the "multitarget hypothesis" (39).

There are several published examples of multitarget cardioprotective strategies directed to distinct signaling pathways within the cardiomyocyte (**Table 1**). For example, in a pig AMI model, the combination of limb RIC with either GIK or exenatide (a glucagon-like peptide-1 mimetic) at the time of reperfusion reduced infarct size to a greater extent than either intervention alone (8). Importantly, the interventions were shown to have distinct intracellular targets, with RIC decreasing oxidative stress (myocardial nitrotyrosine levels) and endothelial NOS (eNOS) uncoupling, and GIK and exenatide shifting cardiac metabolism toward increased glycolysis (8).

TABLE 4 Experimental Studies Illustrating the Potential for Additive Cardioprotection With Multiple Cardioprotective Agents Or Interventions Targeting the Same Signaling Pathway but With Potentiating Effects*

First Author, Year (Ref. #)	Experimental AMI Model	Cardioprotective Agents or Interventions	Cardioprotective Effect	Signaling Pathways
Xin et al., 2010 (62)	In vivo rat	Limb RIPerC (4 × 5/5-min cycles) IPost (6 × 10/10-s cycles)	Additive effects on reducing MI size, reducing ROS at reperfusion and inhibiting apoptosis	Additive effects on Akt and Erk1/2 phosphorylation
Huang et al., 2011 (63)	In vivo rat	Esmolol infusion at reperfusion Milrinone infusion at reperfusion	Additive effects on reducing MI size, and inhibiting apoptosis	Additive effects on PKA activity and Akt phosphorylation
Fan et al., 2012 (51)	In vivo diabetic and nondiabetic rats	Atorvastatin at reperfusion IPost (6 × 10/10 s)	Diabetic heart resistant to IPost and partially protected by atorvastatin. However, combination of IPost and atorvastatin reduces MI size	Additive effects on Akt and eNOS
Tratsiakovich et al., 2013 (46)	In vivo rat and pig	L-arginine at reperfusion Tetrahydrobiopterin (BH_4) at reperfusion	Additive effects on reducing MI size, and reducing ROS at reperfusion	Additive cardioprotection mediated by NOS
Wang et al., 2015 (64)	In vivo rat	Limb RIPerC (1 cycle of 10-min ischemia and 5-min reperfusion) Vagal stimulation	Additive effects on reducing MI size and inflammation	Not investigated

*Other criteria are as per Table 1.
NOS = nitric oxide synthase; PKA = protein kinase A; other abbreviations as in Tables 1 and 3.

MULTIPLE CARDIOPROTECTIVE AGENTS OR INTERVENTIONS WITH NONCARDIOMYOCYTE TARGETS. Combining cardiomyocyte-targeted therapies with therapies that target noncardiomyocyte components in the heart (e.g., those improving tissue perfusion) may provide a more effective strategy for cardioprotection (Table 2). One example is provided by $P2Y_{12}$ inhibitors (such as ticagrelor and cangrelor), which are known to reduce infarct size (40). Because all patients with AMI receive a $P2Y_{12}$ receptor antagonist, a cardioprotective agent, to be effective, must provide additive protection on this therapeutic background. In this regard, VX-765, an inhibitor of caspase 1-mediated pyroptosis, has been demonstrated in rats to provide such an additive benefit on a therapeutic background of the $P2Y_{12}$ inhibitors ticagrelor or cangrelor (41). In another example, targeting necroptosis with necrostatin-1 and apoptosis (presumably in nonmyocytes) with Z-VAD during ischemia and reperfusion conferred additive reduction in infarct size in isolated perfused guinea pig hearts (42).

There would appear to be a solid rationale for combining one agent targeting the microcirculation (MVO) with another targeting cardiomyocytes. Unfortunately, there has been only marginal success to date in trying to relieve MVO and improve microcirculatory flow after MI, even experimentally. Some of the more promising candidates include the vasoactive compounds adenosine and NO (32). Recombinant human angiopoietin-like protein 4 (ANGPTL4) indeed reduced infarct size and prevented no-reflow and intramyocardial hemorrhage in mice (43).

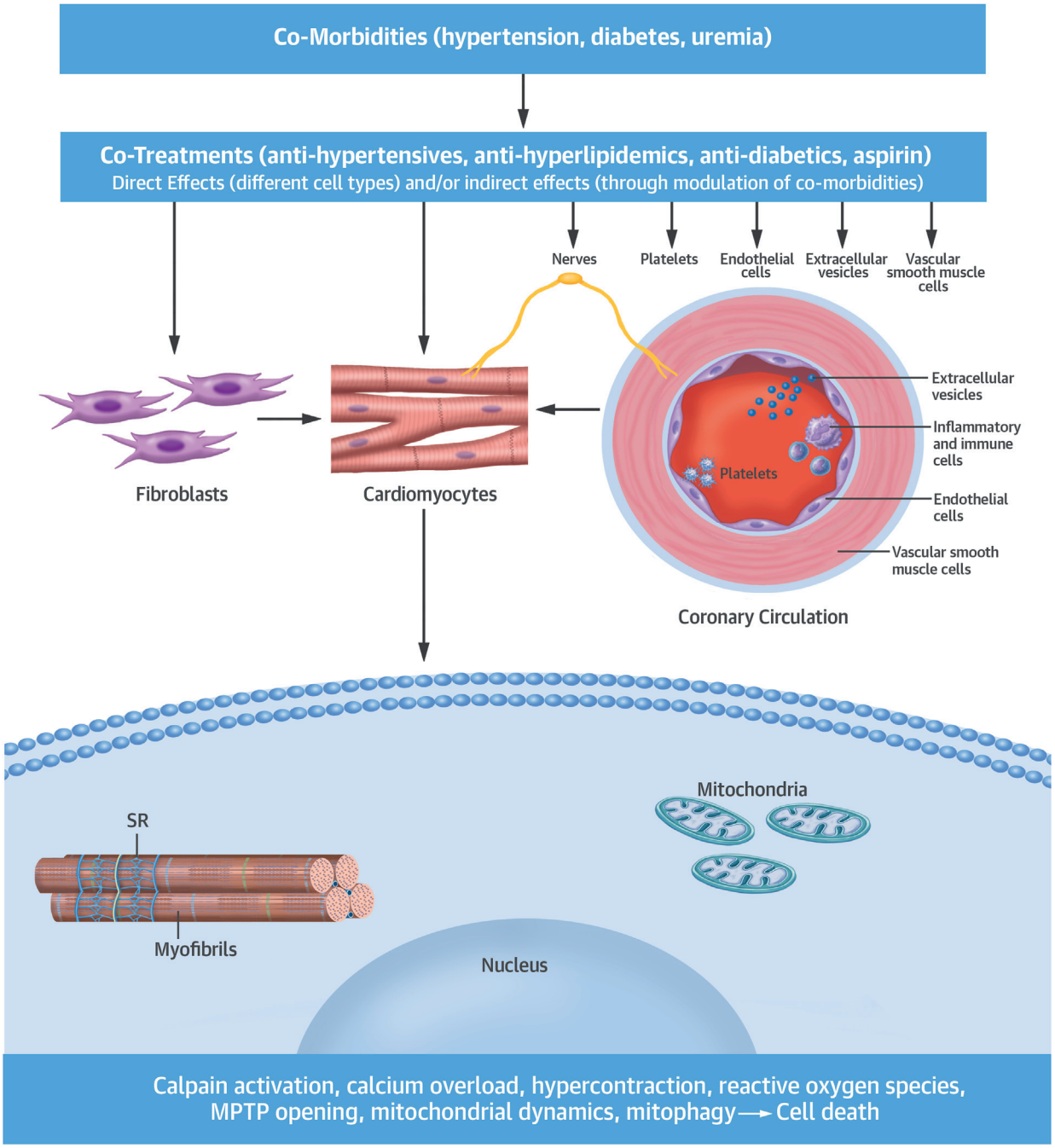
MULTIPLE CARDIOPROTECTIVE AGENTS OR INTERVENTIONS TARGETING DISTINCT TIME-POINTS DURING ISCHEMIA AND REPERFUSION. Given the possibility of intervening at 3 different time points in the setting of AMI (i.e., during ischemia, at reperfusion, and late into reperfusion), there is an opportunity to combine 2 or more cardioprotective therapies to target these 3 different phases in order to achieve additive cardioprotection (Table 3). An excellent example of this multitarget cardioprotective approach was provided by Yang et al. (44), who

TABLE 5 Experimental Studies Illustrating the Potential for Additive Cardioprotection With Multiple Cardioprotective Agents or Interventions Having Multiple Targets*

First Author, Year (Ref. #)	Experimental AMI Model	Cardioprotective Agents or Interventions	Cardioprotective Effect	Signaling Pathways
Rastaldo et al., 2012 (48)	Isolated rat heart	Hybrid molecule containing NO donor and antioxidant	Additive effects on reducing MI size	Mitochondrial K_{ATP} channel
Lougiakis et al., 2016 (47)	In vivo rabbit	Hybrid molecule containing H_2S-donor and adenosine analogue at reperfusion	Additive effects on reducing MI size	cGMP/PKG/phospholamban pathway
García-Ruiz et al., 2016 (17) and Garcia-Prieto et al., 2017 (27)	In vivo pig In vivo mouse	Intravenous metoprolol targeting simultaneously cardiomyocytes (reducing energy demand), and neutrophils (inhibiting migration and neutrophil–platelet coaggregates)	Reduces infarct size when given at different times of ischemia duration, but effect is stronger when given earlier	Cardiomyocyte oxygen consumption reduction and neutrophil conformational re-arrangements

*Other criteria are as per Table 1.
cGMP = cyclic guanosine monophosphate; PKG = protein kinase G; other abbreviations as in Table 1.

FIGURE 1 Main Cardioprotective Signaling Pathways That Can Be Targeted in Combination



Different comorbidities and cotreatments can influence protection via effects on different cellular and subcellular targets, as indicated by **arrows**.
MPTP = mitochondrial permeability transition pore; SR = sarcoplasmic reticulum.

showed that triple therapy combining mild hypothermia and cariporide (a sodium-hydrogen exchanger inhibitor) during ischemia, with cangrelor added at reperfusion conferred additive protection.

MULTIPLE CARDIOPROTECTIVE AGENTS OR INTERVENTIONS TARGETING THE SAME SIGNALING PATHWAY BUT WITH ADDITIVE EFFECTS. In some situations, 2 agents may act on the same signaling

TABLE 6 Main Clinical Studies (Completed and Ongoing) Investigating Multitargeted Agents or Interventions Against AMI IRI in STEMI Patients Undergoing PPCI

First Author, Year (Trial) (Ref. #)	No. of Patients (Control/Intervention)	Multitargeted Treatment Intervention	Approach	Primary Endpoint	Outcome
Completed studies					
Eitel et al., 2015 (LIPSIA-COND) (54)	Control/IPost/RIPerC+IPost 232/232/232	Combined limb RIPerC + IPost	RIPerC: In hospital upper limb 3 cycles (5/5 min, 200 mm Hg), IPost: (1-min balloon inflation/1-min deflation) started as soon as possible after reopening of the culprit coronary artery	Myocardial salvage index (edema and late gadolinium enhancement by CMR)	23% increase in salvage index No limb RIPerC alone group
Pasupathy et al., 2017 (NACIAM) (56)	IV GTN/IV GTN+NAC 38/37	Combined NAC+GTN	IV GTN: IV NAC:	MI size (late gadolinium enhancement by CMR)	5.5% reduction in infarct size All patients received GTN
Actively recruiting studies					
Ovize et al., (CARIOCA) (NCT03155022)	Estimated enrolment 355/355	Combined limb RIPerC and IPost	RIC: In-hospital, upper limb, 4 cycles (5/5 min, 200 mm Hg) initiated as soon as possible before PCI IPost: 4 cycles (1 min balloon inflation/ 1 min deflation) started as soon as possible after reopening of the culprit coronary artery	Combined incidence of all-cause mortality; worsening of heart failure during initial hospitalization or rehospitalization for heart failure at 6 months after PPCI	Recruiting
Garcia-Dorado et al., COMBAT-MI (NCT02404376)	2 × 2 factorial design (RIC, exenatide, both, or neither) 107/107/107/107	Combined limb RIPerC+ exenatide	RIC: in-hospital, upper limb, 4 cycles (5/5 min, 200 mm Hg) Intravenous infusion of exenatide initiated before reperfusion	Myocardial infarct size (late gadolinium enhancement by CMR)	Recruiting

CARIOCA = Combined Application of Remote and Intra-Coronary Ischemic Conditioning in Acute Myocardial Infarction; CMR = cardiac magnetic resonance; COMBAT-MI = COMBinAtion Therapy in Myocardial Infarction; GTN = nitroglycerin; IRI = ischemia-reperfusion injury; IV = intravenous; LIPSIA-COND = Effect of Conditioning on Myocardial Damage in STEMI; NAC = *N*-acetylcysteine; NACIAM = N-acetylcysteine in Acute Myocardial Infarction; PCI = percutaneous coronary intervention; PPCI = primary percutaneous coronary intervention; RIC = remote ischemic conditioning; STEMI = ST-segment elevation myocardial infarction; other abbreviations as in Tables 1 and 3.

pathway, one potentiating the other's cardioprotective effects (Table 4). For example, coadministration of the NOS substrate L-arginine and cofactor tetrahydrobiopterin (BH_4) just before reperfusion significantly reduced MI size in both rats and pigs, despite neither being protective on their own (45,46).

A SINGLE CARDIOPROTECTIVE AGENT OR INTERVENTION WITH MULTIPLE TARGETS. There are many examples of a single cardioprotective agent or single intervention having multiple targets (Table 5), and one would intuitively expect that these therapies would be more effective than a single-target agent or intervention. For example, intravenous metoprolol administered before reperfusion reduces both infarct size and MVO in mice (27), pigs (17), and humans (13). Classically, metoprolol has been considered to reduce ischemic injury by reducing energy demands from cardiomyocytes, because it is more effective when given early during ischemia (17). However, metoprolol has recently been shown to act via the β1 adrenergic receptors on neutrophils to decrease neutrophil-platelet coaggregate formation during reperfusion (17), which can explain the strong effect of metoprolol on MVO. The dual-target benefits of metoprolol appear to be specific to this drug and not a class effect.

The endogenous cardioprotective strategies of IPC, IPost, and RIC are known to protect the heart through a number of different signaling pathways and might therefore be assumed to confer a stronger cardioprotective effect than a single-target agent.

A single miRNA or small interfering RNA may protect the heart against acute IRI through its effects on a variety of different target mRNAs. Hybrid molecules may have 2 or more structural domains acting as 2 distinct pharmacophores to provide additive cardioprotection. For example, a hybrid compound that combines the adenine nucleus with a moiety that slowly releases hydrogen sulfide (H_2S)-induced additive cardioprotection (47). A hybrid molecule consisting of a lipophilic NO donor and a lipophilic antioxidant compound protected the rat heart against acute IRI if given as a hybrid molecule, but not as a mixture (48).

THE IMPACT OF COMORBIDITIES AND COTREATMENTS

Since the first observations in animal studies in the late nineties, it has been well established that many of the signaling pathways involved in the protection by ischemic conditioning interventions are affected by several cardiovascular risk factors and comorbidities such as sex, age, hypertension, and metabolic diseases such as hyperlipidemia and diabetes (5). For

example, conditioning stimuli are less effective in diabetic animals because they are less able to activate PI3K/Akt (49). A stronger stimulus or combination strategy against additional targets may be required to fully activate the protective pathways. For example, cardioprotection can be restored by administering an inhibitor of phosphatase and tensin homolog (PTEN)—a major negative regulator of PI3K/Akt—to maximize PI3K/Akt activation (50). Medications used to treat a comorbidity may either interfere or enhance cardioprotective signaling (6). Atorvastatin at reperfusion combined with IPost was able to overcome the resistance of the diabetic murine heart to cardioprotection by augmenting the activation of the Akt-eNOS pathway (51).

Most studies showing the interaction of certain comorbidities with cardioprotection have been performed in single comorbidity models with no specific treatment of the comorbidity. Although most animal experiments on IRI and protection from it were performed in young and otherwise healthy (therefore untreated) animals, patients recruited into clinical cardioprotection trials are usually of advanced age and have numerous comorbidities and related comedications as well as short-term treatments related to AMI. An important example of comedications confounding cardioprotection are anesthetics including propofol that can affect cardioprotection (6). Therefore, more studies in adequate animal models more closely mimicking the clinical situation with multiple comorbidities and related comedications would be ideal for finding drug targets (5). In this regard, it should be noted that existing clinical therapies post-MI already consist of many combination strategies.

The impact of comorbidities and cotreatments on cardioprotection has long been suspected; however, subgroup analyses performed in largescale clinical studies including patients with multiple comorbidities and comedications have not confirmed the confounding effect of a particular single comorbidity or comedication (e.g., in the CIRCUS [Cyclosporine and Prognosis in Acute Myocardial Infarction (MI) Patients] trial on cyclosporine A [52]).

CLINICAL STUDIES OF MULTITARGET THERAPY. The main target patient population for cardioprotection is those with STEMI undergoing immediate revascularization by PPCI. Current clinical studies of multitarget therapies are limited to combinations of different ischemic conditioning strategies, a combination of pharmacological treatments, or a combination of pharmacological and conditioning strategies, (**Table 6**), whereas physical measures such as hypothermia or nerve stimulation have not been studied in combination with other cardioprotective strategies.

As an example of a study investigating 2 interventions targeted primarily to cardiomyocytes, exenatide (53) and RIC (15), which have each demonstrated cardioprotective efficacy individually in STEMI patients undergoing PPCI, are being investigated in combination in the COMBAT-MI (COMBinAtion Therapy in Myocardial Infarction) trial (NCT02404376).

In an investigation of 2 therapies administered at different time points, Eitel et al. (54) studied the combination of in-hospital RIC before reperfusion and intracoronary IPost after reopening the culprit coronary artery in 696 STEMI patients. Whereas IPost alone failed to improve myocardial salvage index assessed by cardiac magnetic resonance, combined RIC and IPost increased the salvage index. Because there was no group treated with RIC alone, the study could not confirm an additive effect. Another clinical study failed to observe an additive cardioprotective effect with limb RIC and IPost (55). The CARIOCA (Combined Application of Remote and Intra-Coronary Ischemic Conditioning in Acute Myocardial Infarction) trial (NCT03155022), investigating the efficacy of combined in-hospital RIC before reperfusion and IPost on clinical outcome is ongoing.

In a study intended to test the potentiating effect of 2 different cardioprotective agents, the NACIAM (N-acetylcysteine in Acute Myocardial Infarction) trial (56) examined the effects of *N*-acetylcysteine (NAC) on infarct size in 75 patients with STEMI undergoing PPCI. NAC is an antioxidant and potentiates the effects of nitroglycerine. With background nitroglycerin infusion administered to all, patients receiving NAC had an absolute 5.5% reduction in cardiac magnetic resonance imaging–assessed infarct size relative to placebo. However, the study design of the trial did not provide conclusive information about mechanisms involved, because all patients received nitroglycerin (56).

As an example of a single agent targeting multiple pathways, metoprolol has been studied in STEMI patients; however, results have been mixed (13,57).

FUTURE RECOMMENDATIONS

Extensive evidence accumulated over the past 30 years has shown that a multitude of cardioprotective therapies are effective at reducing infarct size in animal models of IRI (3,4,6,32). However, routinely used animal models of IRI do not adequately recapitulate the complex phenomenon of IRI in patients. Here, we hypothesize that to be effective in these models, and to effectively translate cardioprotection to patients, a multitarget cardioprotective therapy is necessary. Combinations of interventions with solid preclinical

information on mechanism of action, efficacy, and safety, and that are easily applicable are good candidates to be moved to clinical trials. In designing such a trial, a factorial design may be used to prove additive benefit of a combination, but this approach increases patient numbers needed. A better approach may be to first prove additive benefit in animal models and then test the combination in patients against control. Another important consideration is that STEMI patients receive comedications such as $P2Y_{12}$ inhibitors. Other factors such as the effect on arrhythmias and long-term cardiac remodeling should also be considered.

In light of the examples discussed in the previous sections, some promising examples of approaches to multitargeted cardioprotection include:

- A combination of RIC with a drug with a different mechanism of action—this is being tested in the COMBAT-MI trial.
- A combination of a drug that activates endogenous cardioprotective pathways (RISK, SAFE, cGMP/PKG) with a drug that inhibits cell death pathways.
- A drug targeting vascular injury/inflammation with a drug targeting cardiomyocyte death.

We hypothesize that the ideal multitargeted therapy might be one that can target MVO (e.g., intravenous cangrelor or ANGPL4), target cardiomyocytes (e.g., remote ischemic per-conditioning) and target inflammation (e.g., metoprolol). The timing of administration of these modalities could potentially be separated over time.

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