Moving forwards by blocking back-flow: the yin and yang of MI therapy

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Abstract

Mitochondrial reactive oxygen species (ROS) production has emerged as an important pathological mechanism in myocardial ischemia/reperfusion (IR) injury. Attempts at targeting ROS by scavenging using antioxidants have however been clinically disappointing. This review will provide an overview of the current understanding of mitochondrial ROS in IR injury. We will outline novel therapeutic approaches designed to directly target the mitochondrial respiratory chain and prevent excessive ROS production and its associated pathology. This approach could lead to more effective interventions in an area where there is an urgent need for new treatments.

Keywords: mitochondria, reactive oxygen species, ischemia/reperfusion injury.

Introduction

Mitochondria are an important source of reactive oxygen species (ROS) in mammalian cells and play a critical role in cardiac function. Under physiological conditions, low levels of ROS are produced as a by-product of mitochondrial respiration and act as essential cellular mediators in a variety of biological processes including regulation of the immune response and autophagy[1–3]. Stress or injury can however cause ROS to increase significantly, overwhelming endogenous antioxidant mechanisms and resulting in severe oxidative damage to cellular components such as lipids, proteins and DNA[4]. Mitochondrial ROS are now known to be key mediators of mitochondrial dysfunction and disease pathology in a range of cardiovascular conditions including atherosclerosis, cardiac hypertrophy, chronic heart failure, ventricular remodeling and ischemia/reperfusion (IR) injury[5–7]. Upon reperfusion of ischemic myocardium, the rapid re-introduction of oxygen into the cell leads to a burst of ROS generation that triggers opening of the mitochondrial permeability transition (mPTP) pore and myocardial cell death. Significant progress has been made in the field of inhibiting or scavenging ROS in an attempt to preserve mitochondrial and cardiomyocyte function. However despite the large body of evidence supporting the inhibition of oxidative stress as a valuable therapeutic strategy, treatment with antioxidants has failed to deliver clinically significant benefits[8]. In the present review we will discuss the role of mitochondrial ROS in cardiac IR injury, describing the current mechanisms that are thought to drive its production. Furthermore we will highlight current methods at targeting mitochondria ROS production with a particular focus on interventions that inhibit complexes I and II.

Ischemia/reperfusion injury

IR injury remains a leading cause of death worldwide and the primary cause of chronic heart failure (CHF). While the past few decades have seen a marked improvement in outcomes in patients treated with early reperfusion therapy, currently one in four patients will die or present with heart failure within one year post-injury[9]. Reperfusion of the ischemic myocardium is essential in order to salvage viable tissue but paradoxically the rapid restoration of blood flow can induce injury beyond that of the initial ischemic insult. Known as reperfusion injury, studies have shown that it can account for up to 50% of the total tissue damage[7] for which there is currently no effective therapy available in the clinic. The mechanisms underlying IR injury are multifactorial and have been extensively reviewed elsewhere[7,10]. However, it is generally accepted that mitochondrial dysfunction is central to the pathology of both IR injury and CHF with the mitochondrion not only being the main producer of ROS but also a primary target of ROS damage.

Cardiac metabolism is predominantly aerobic. As such the maintenance of normal cardiac function and viability is highly dependent on the constant delivery of oxygen. During periods of severe myocardial ischemia profound disturbances in metabolism occur resulting in a shift towards anaerobic glycolysis. ATP depletion and lactic acidosis drive cytosolic sodium accumulation via the sodium/hydrogen exchanger and as a consequence excess Na^+ is extruded through the reverse action of the plasma membrane sodium/calcium exchanger[11]. Typical calcium (Ca^{2+}) management by the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) is prevented, due to depletion of mitochondrially-derived ATP, resulting in cytosolic Ca^{2+} overload. Furthermore, there is an accumulation of metabolic end-products, including hypoxanthine, xanthine and succinate[12–14], and the formation of pro-inflammatory mediators that promote the infiltration and activation of neutrophils. All these events are thought to 'prime' the heart for the large burst of ROS generation upon reperfusion. Re-oxygenation of the cell at reperfusion and rapid restoration of the mitochondrial membrane potential ($\Delta\Psi_m$) results in a large Ca^{2+} influx into mitochondria. Together with a burst of ROS production [15] and normalization of pH[16], opening of the mPTP is induced[17]. The prolonged opening of the mPTP is now generally agreed to be decisive in committing cells to death upon reperfusion. The mPTP is a highly conducting channel in the mitochondrial inner membrane. While the exact nature of the pore is under debate, recent evidence suggests that the F_oF_1-ATP synthase is a major component[18,19]. While the low pH present during ischemia

prevents formation of the mPTP, the normalization of pH at reperfusion results in mPTP formation and subsequent collapse of $\Delta\Psi_m$, cytochrome c release, ATP depletion and cellular death. Opening of the mPTP is therefore a critical component in reperfusion injury pathology[10,19–21].

The mitochondrial electron transport chain is an important source of ROS during IR injury but several other sources can also contribute. They include monoamine oxidase (MAO) on the surface of the mitochondrial outer membrane, xanthine oxidases, NAD(P)H oxidases, and uncoupled nitric oxide synthases[22–24]. The contribution of these enzymes to total IR induced ROS production is however thought to be lower than that of mitochondria and to occur later in the IR injury process, so will not be discussed further in this review.

The mitochondrial respiratory chain

Since this review is aimed at a general audience, a brief primer on mitochondrial respiratory activity is provided here, and is illustrated in Figure 1. Substrates (pyruvate from glycolysis or acetyl-CoA from β-oxidation) are decarboxylated in the tricarboxylic acid (TCA) cycle to yield reducing equivalents NADH and $FADH_2$. Electrons are then passed onto complexes I or II respectively, and then to the mobile electron carrier co-enzyme Q_{10} (ubiquinone), reducing it to ubiquinol. Ubiquinol is re-oxidized by complex III, passing electrons to cytochrome c then cytochrome oxidase and finally oxygen, generating H_2O. The respiratory complexes are electron-driven proton pumps, such that this passage of electrons is coupled to the generation of a trans-membrane proton electrochemical potential gradient (positive outside). The electrochemical energy in this gradient is then used by the F_oF_1-ATP synthase to generate ATP. It is important to note that the sharing of Co-Q as a common electron carrier by complexes I, II, and III is what permits these complexes to exist in multiple configurations, such that electrons can flow from I to III, II to III, I to II, and II to I, as is shown in Figure 2.

Central role of mitochondria

Mitochondria are essential organelles for normal cellular function. Occupying up to 30% of total cardiomyocyte volume, they are the main source of ATP for the contracting cell through oxidative phosphorylation[25]. Mitochondria are also a key source of cellular ROS production with oxygen being converted by mitochondria to superoxide at complexes I and III[26], however while the amounts of superoxide produced by isolated mitochondria can be readily estimated, the amount produced in vivo and the factors that regulate this production remain obscure. The superoxide

produced in the mitochondrial matrix is then largely dismutated to hydrogen peroxide (H_2O_2) by manganese superoxide dismutase (Mn-SOD)[27]. While several other sources of ROS within mitochondria have been documented (e.g. α-ketoglutarate dehydrogenase, monoamine oxidase, ETF-QOR of β-oxidation, α-glycoerophosphate dehydrogenase[28,29], their relative importance under *in vivo* conditions is poorly understood. Thus, the rest of this review will focus primarily on complex I, as this appears to be quantitatively the most important source of ROS in the setting of ischemia reperfusion injury[4].

In the context of IR pathology elevated mitochondrial ROS levels drive oxidative damage to mitochondria, which results in disruption of the respiratory machinery and ATP generation. Additionally, in conjunction with dysregulated calcium levels, mitochondrial ROS lead to induction of the mPTP, contributing to both apoptotic and necrotic cell death due to IR. Because of the central role of mitochondrial ROS in IR pathology, many investigations have focused on characterizing the pathways that underlie their generation. In the past decade such studies have increasingly highlighted a central role for mitochondrial complex I as the most significant superoxide source during IR[30,31]. More recently, it has been shown that generation of superoxide from complex I during IR is dependent on electron supply from the mitochondrial citric acid cycle (CAC) intermediate succinate[14]. Succinate, which accumulates significantly during ischemia through the reverse action of complex II, is rapidly oxidized in the first minutes of reperfusion. This rapid oxidation drives reverse electron transport (RET) at complex I, in which electrons are forced from reduced Coenzyme Q (CoQ) back to complex I generating large amounts of superoxide. This process can be described as a 'yin-yang' formation in which during ischemia QH_2 generated by complex I working forward, is oxidized by complex II working in reverse. At reperfusion, complex II acting in forward mode consumes the accumulated succinate driving RET at complex I (Figure 2). Most interestingly, from a therapeutic perspective, it has been shown that this generation of damaging ROS upon reperfusion can be inhibited, either by preventing the accumulation of succinate during ischemia, or by inhibiting the succinate-dependent superoxide production by transient inactivation of complex I[14,32]. Both approaches will be discussed further below.

"Good" versus "bad" ROS

An intriguing aspect to ROS production in the heart is that depending on the circumstances and context, it can be considered either “good” or “bad”. That is, not all amounts of ROS are damaging and only when levels reach beyond the capacity of endogenous antioxidant mechanisms will ROS become detrimental to cell function and contribute to IR pathology. Conversely ROS production has also been found to be a trigger for protection against IR injury particularly through the activation of survival programs during ischemic pre- (IPC) and post-conditioning (IPost)[33,34]. IPC, first demonstrated by Murray in 1986[35], is a phenomenon in which brief cycles of IR protect the heart from reperfusion injury after a prolonged ischemic insult. ROS generated from these IR cycles are recognized as triggers for a cascade of signaling events that result in reduced tissue damage with the mitochondrion considered a primary source[36]. Pre-treating isolated rabbit hearts with oxygen radicals can reproduce the beneficial effect of IPC on infarct size[37] while giving ROS scavengers prior to ischemia abolishes IPC-induced protection[34]. The most straightforward interpretation of this intriguing observation is that while low levels of ROS can be beneficial by up-regulating protective mechanisms, a larger amount of ROS amount has detrimental effects. This counter-balance between ‘good’ and ‘bad’ levels, known as “mitohormesis”[2], is supported by an increasing body of work in which low levels of ROS are thought to act as signaling molecules to promote health and extend lifespan[2]. In the context of IR injury a small increase in ROS, sufficient to lead to transient mPTP opening, has been shown to be protective against subsequent IR injury[38]. On the other hand, prolonged ROS exposure leading to sustained mPTP opening inevitably leads to irreversible mitochondrial damage and ultimately cell death. The threshold at which ROS production transitions from being protective to becoming harmful may be modulated by a variety of factors such as diabetes, sex and age; risk factors which are already established to affect the efficacy of cardioprotective strategies[39]. For example, one way in which sex may determine the mPTP response to ROS, is in the levels of nitric oxide (NO). It is known that eNOS is regulated by estrogen[40], and this may directly impact ROS levels, in addition to NO being a direct inhibitor of the pore[41]. Similarly for aging, the sensitivity of the Keap1/Nrf2 signaling axis, a key genetic response to oxidative stress, is known to decline with age[42].

Another hypothesis explaining the protean roles of ROS could be ascribed to the spatial distribution of its sites of production. It is well established that for many signaling pathways the intracellular location of the signal plays a crucial role, for example the compartmentalization of the cGMP – guanylate cyclase pathway[43]. Unfortunately, the details of the localization of ROS signaling are difficult to assess *in*

vivo. However, given that different classes of mitochondria exist in the heart (subsarcolemmal versus intrafibrillar populations) behave differently during ischemic pre- and post-conditioning[44,45], it seems likely that the spatial distribution of mitochondrial ROS generation may also be a key variable. Finally, the timing of ROS generation could be important during IR with ROS being beneficial as a trigger of preconditioning-like signaling before a prolonged period of ischemia, while the large ROS burst at reperfusion induces many detrimental downstream effects.

Therapeutic implications: Preventing excessive ROS generation

The compelling body of evidence linking reperfusion-induced ROS production to cardiac pathology has not surprisingly led to the testing of a wide range of anti-oxidant approaches to mitigate the detrimental effects of oxidative stress upon reperfusion. While many antioxidant strategies have shown benefit when applied to *in vitro* and *in vivo* model systems, only a tiny fraction has translated to improvements in major clinical end-points in human trials[46]. For example, antioxidants including Vitamin C, Vitamin E, Edavarone and Coenzyme Q10 have shown disappointing or conflicting outcomes in patients[8]. Many possible reasons for these poor results have been considered; the dosage of drug may not be optimal to achieve sufficient myocardial levels at reperfusion, the timing of the intervention in relation to the onset of ischemia or point of reperfusion may be incorrect and pre-clinical models used may not be appropriate for screening new compounds for human use[47,48]. The development of mitochondria-targeted antioxidants in which compounds are localized to the mitochondrion by conjugation to a triphenylphosphonium (TPP^+) cation may address some of difficulties inherent in using un-targeted antioxidants that do not accumulate in mitochondria[49]. MitoQ is a TPP^+ modified ubiquinol that upon delivery to mitochondria decreases oxidative damage and has been shown to be protective against both cardiac[50] and liver IR injury[51] *in vivo* as well as protecting against oxidative damage in a murine model of heart transplantation[52]. The potential benefit of these targeted compounds against IR injury in humans has yet to be determined. A further consideration is that a more effective strategy may be to block the excessive ROS production that occurs upon reperfusion at its source, rather than scavenge it after it has been produced. Moreover, this approach could in principle allow the blockade of ROS production only when it becomes pathological, avoiding the potential disruption to cellular homeostasis by altering physiologically important cellular signals by “good” ROS through chronic antioxidant treatment. Given that the mitochondrial respiratory chain is a critical source of ROS upon reperfusion, it has become a major target for novel

compounds aimed at ameliorating IR injury and this strategy will be discussed in the next section.

Pharmacologic inhibitors of the respiratory chain as therapeutics for IR injury.

Despite the lack of oxygen during ischemia or hypoxia leading to inhibition of the respiratory chain, a wide variety of respiratory inhibitors have been demonstrated to afford protection against IR injury. Table 1 lists several such inhibitors and their sites of action within the respiratory chain. Until recently, it was thought the mechanism of action for these respiratory inhibitors was centered around the "gradual wake up" hypothesis of reperfusion therapy[53]. In this paradigm, a rapid reestablishment of respiratory activity at reperfusion leads to a surge of mitochondrial Ca^{2+} uptake and ROS generation which contribute to mPTP opening. It was hypothesized that the wash-out of a respiratory inhibitor present at reperfusion would permit a more gradual wake up of metabolism, thus avoiding these pathogenic effects. However, the recent identification of the source of ROS at reperfusion, namely the reverse electron transfer at complex I, forces a further focusing of this paradigm[14]. Namely it cannot go un-noticed that ~85% of the protective respiratory inhibitors listed in Table 1 act at the level of complex I or II. While the prevalence of agents hitting a given pharmacologic target cannot be taken as evidence of the central biological importance of the target, it is notable that there are well known inhibitors of other parts of the respiratory chain (e.g. cyanide for complex IV, myxothiazol for complex III) that have not been found useful in a therapeutic setting. Furthermore, while many of the molecules in Table 1 act at a pleiotropic level, there are some exquisitely specific drugs targeted at complexes I and II (e.g. rotenone and atpenin A5), which are most likely mediating their effects via these complexes and not through off-target mechanisms. We will now discuss these complexes in turn and the current evidence for their modulation in protecting the myocardium during IR injury.

Complex I

Complex I (NADH ubiquinone oxidoreductase) is the primary point of electron entry within mitochondria responsible for the oxidation of NADH, derived from glycolysis, the CAC and the β-oxidation of fatty acids. Complex I transfers electrons to CoQ and protons are transported across the inner membrane contributing to the mitochondrial proton motive force. In addition it is an important site for ROS generation with complex I producing large amounts of superoxide in the presence of a high $NADH/NAD^+$ ratio where oxygen reacts with a fully reduced flavin mononucleotide

(FMN) site[4]. Complex I can also produce a large amount of ROS during RET where, in the presence of a highly reduced CoQ pool and a close to maximal proton motive force, electrons are pushed backward from $CoQH_2$ through complex I reducing NAD^+ to NADH and also producing superoxide[54,55]. While the physiological relevance of RET *in vivo* is only now being elucidated it produces the largest rate of mitochondrial ROS production known to occur within mitochondria. Furthermore, this process of superoxide production by RET at complex I seems to be the major source of ROS early during IR injury[14].

During prolonged ischemia, when complex I is not oxidizing NADH due to the lack of oxygen, the protein converts to a 'deactive' state[56,57]. Reperfusion of the tissue results in the rapid re-activation of complex I and the generation of large amounts of cytotoxic ROS by RET[14]. Inhibitors of complex I including rotenone[31] and amobarbital[58] have found to be protective when given during cardiac IR injury indicating that preventing the reactivation of complex I upon reperfusion is a promising potential therapeutic strategy. Of course, the use of irreversible complex I inhibitors is not viable as a therapy, but interestingly when complex I undergoes the 'deactive' transition a critical cysteine, cysteine 39 on the ND3 subunit becomes exposed to modification[56,57]. This residue can be reversibly inhibited by its *S*-nitrosation by *S*-nitrosothiols such as SNO-MPG[59] or MitoSNO[32]. Further supporting a role for this cysteine residue in cardioprotection, recent work has shown that damage protection during IR, IPC and IPost correlates highly with the persistent *S*-nitrosation of mitochondrial protein thiols, with complex I as a chief target[32,60–62]. One example of this protective mechanism is MitoSNO, a mitochondria-targeted drug that prevents ROS production from complex I during early reperfusion following IR injury[32]. MitoSNO is a mitochondria-targeted *S*-nitrosothiol based on the NO donor *S*-nitroso-N-acetylpenicillamine (SNAP) coupled to the TPP^+ cation which leads to its rapid, several hundred-fold accumulation, driven by both the plasma and mitochondrial membrane potentials, into the mitochondrial matrix where it accumulates within minutes of intravenous injection[63,64]. Upon uptake into mitochondria MitoSNO reacts rapidly with intra-mitochondrial thiols and *S*-nitrosates cysteine 39 on subunit ND3 of complex I "locking" the enzyme in its de-active form at reperfusion and thereby preventing the excessive burst of ROS upon reperfusion[32]. The modification is reversed with a half-life of ~5 min by the endogenous mitochondrial glutathione and thioredoxin systems, allowing complex I to return to full levels of activity a few minutes after reperfusion[14]. Our studies have shown that MitoSNO not only protected against IR injury *in vivo*[32] but also greatly enhanced long-term cardiac function post-IR injury[65].

Complex II

Complex II (succinate dehydrogenase) catalyzes the oxidation of succinate to fumarate resulting in the donation of electrons to the respiratory chain via the reduction of FAD to $FADH_2$. Unlike the other respiratory complexes, it does not pump protons across the inner membrane but instead acts to maintain a reduced CoQ pool which has been largely considered to be its primary function[66]. This sequence also creates a direct link between two major mitochondrial pathways, the CAC and the respiratory chain. Several roles for complex II have however also been recently proposed that expand beyond this with evidence now for direct complex II-mediated ROS generation[67] as well as a mechanistic link with the putative mitochondrial ATP-sensitive potassium channel (mtK_{ATP})[68]. Complex II is also now recognized as a key modulator of mitochondrial ROS production by other respiratory complexes, particularly complex I.

The accumulation of excessive ischemic succinate, via the reverse action of complex II, is considered a critical driver of ROS formation at reperfusion. Preventing either its build-up during ischemia or its rapid oxidation at reperfusion are therefore potential valuable therapeutic strategies to reduce detrimental ROS generation and protect against IR injury. In agreement with this an extensive body of work exists demonstrating the inhibition of respiration at complex II can decrease ROS production[69]. Inhibitors such as dimethyl malonate, diazoxide and atpenin A5 all protect against IR injury when given prior to ischemia[14,70–72]. Moreover protection afforded by dimethyl malonate *in vivo* was attributed directly to the attenuation of ischemic levels of succinate and inhibition of mitochondrial ROS generation at reperfusion[14]. There is also some evidence that malonate itself may act as an endogenous protector against IR with the compound being generated endogenously in mitochondria under conditions mimicking IPC[73]. However whether these compounds exert cardioprotective effects solely via complex II inhibition and ROS generation or if the mtK_{ATP} channel is involved remains a controversially and actively discussed issue. Moreover, while these strategies may be highly useful in situations of predictable ischemia, including elective surgery and organ transplantation, they are not clinically appropriate given patients undergoing an myocardial infarction (MI) arrive at hospital with an already occluded artery. Succinate accumulation during ischemia only becomes pathological upon its rapid oxidation at reperfusion in which it drives RET-mediated ROS production through complex I. By suppressing succinate oxidation at the point of reperfusion through complex II inhibition, compounds such as dimethyl malonate could be potentially cardioprotective. It is therefore essential to

determine if complex II inhibitors are as equally effective at ameliorating cardiac injury when used later in IR such as just prior to reperfusion. Indeed recent work in the isolated mouse heart has demonstrated that the administration of malonate during the first 15 min of reperfusion only was cardioprotective through the inhibition of succinate re-oxidation and the reduction in ROS production and mPTP opening[74]. Whether this important result can be translated to *in vivo* models however remains to be determined.

Future Perspectives & Translational Significance

An important consideration for the potential future use of respiratory inhibition as a therapy for IR injury is the timing of delivery. While clearly the inhibition of complexes I or II at early reperfusion would be anticipated to minimize ROS generation from RET, it is not immediately clear that inhibition of these complexes during ischemia itself would be beneficial. This is because of the yin-yang nature of complexes I and II during ischemia in which complex I continues to operate as a proton pump allowing to some extent the $\Delta\Psi_m$ to be maintained. As such, inhibition of complex I during ischemia may have unforeseen detrimental effects by removing this important function. A further consideration in moving such molecules into a clinical setting is their ease of wash-out, i.e. their tightness of binding to their targets. In the case of rotenone and other tight-binding lipophilic molecules, inhibition would be expected to reverse rather slowly, if at all, while the complex II inhibitor, 3-nitropropionate, is a "suicide inhibitor" that covalently modifies complex II potentially resulting in long term toxic effects on organ function[75]. Furthermore, currently available inhibitors of mitochondrial respiratory complexes are not tissue specific and are therefore present in other important tissues such as the brain. Consequently the chronic delivery of a respiratory inhibitor would be expected to elicit toxic side effects such as neurodegenerative disease. Specifically, long-term inhibition of complex I is associated with Parkinson's disease, complex II Huntington's disease, and complex IV Alzheimer's disease[76,77]. In this regard, another advantage to nitric oxide donors and other short-lived species such as MitoSNO as mitochondrial respiratory inhibitors, is their short time of action and rapid metabolism, which would permit re-establishment of "normal" mitochondrial function once the initial early-reperfusion *danger-period* has passed.

Recently, it has been shown that treatment with a $P2Y_{12}$ inhibitor, such as clopidogrel or ticagrelor, was highly protective in animal models of acute MI as well as in small human studies, and that many conditioning strategies, such as ischemic postconditioning do not offer additional benefits in reducing infarct size[78,79]. This

evidence could very well be the reason for the failures of many recent clinical trials of either ischemic postconditioning or interventions mimicking conditioning. In order to translate any of the above-mentioned compounds targeting complex I or III it is therefore crucial to test whether they have additive effects on top of an effective treatment with $P2Y_{12}$ inhibitors[80]. Further aspects on how to translate preclinical findings into patient care and the challenges especially in acute MI have been extensively reviewed elsewhere[81].

Summary

There is a pressing need for therapeutic approaches to be applied in conjunction with reperfusion therapy to reduce infarction injury and long-term outcome in MI patients. Modulation of the respiratory chain through inhibiting complex I and II are important emerging strategies. These interventions can now be considered as potential rational therapies, arising from the view that the initial burst of ROS from complex I upon reperfusion is due to the accumulation of succinate by the reversal of complex II during ischemia, that then drives the initial burst of ROS at reperfusion by RET at complex I. The reversible inhibition of complexes I and II would therefore prevent this burst of ROS and protect against infarction. Currently, approaches that prevent the accumulation of succinate during ischemia, such as dimethyl malonate, or stabilize the deactive form of complex I by *S*-nitrosation, such as MitoSNO, have been shown to be effective in animal models. Whether these results will translate into the clinic remains to be seen. Certainly the next stages are to see if it is possible to extend and optimize these targets with new and better drugs. However the model of succinate-driven ROS production mediated by complex I and II should facilitate the future development of novel targeted therapies against the generation of excessive mitochondrial ROS in a range of pathologies such as myocardial infarction and stroke.

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Conflicts of Interest

Some of the authors (ETC, MPM, TK) have filed patents in the area of therapies designed to prevent mitochondrial ROS production during cardiac IR injury.

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Site of action	Inhibitor	References
Complex I	Rotenone	31
	Amobarbital	58
	S-nitrosothiols	59,63,82,83
	Nitrite	84,85
	Metformin	86–89
	Capsaicin	90,91
	Isoflurane	92,93
	Ranolazine	94
Complex II	Atpenin A5	72
	Diazoxide	71,95
	Malonate	14,70,73
	Nitroxyl	96,97
	3-nitropropionate	98
	Nitro-alkenes	99
Complex III	Antimycin A	100
Complex IV	Nitric Oxide	101
	Carbon Monoxide	102
	Hydrogen Sulfide	103–105

Table 1 - Mitochondrial respiratory inhibitors that have been shown to protect the heart or brain from IR injury, and their sites of action. Note, some references are paired such that the phenomenon of a molecule inhibiting a respiratory complex, and the phenomenon of it being protective in IR injury, are not necessarily co-observed in the same experimental system. Inclusion of a molecule in this table should not be misconstrued as claiming that the mechanism of its protection is dependent on its effects on a given respiratory complex.

Figure 1.

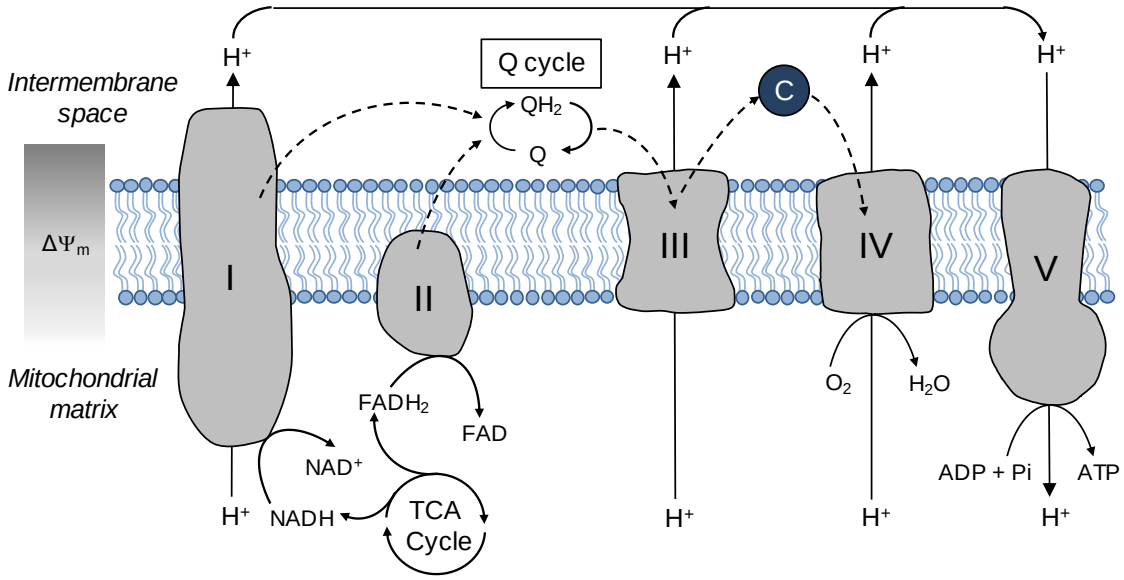

Figure 1. The mitochondrial electron transport chain. Electrons derived from the oxidation of NADH and $FADH_2$ enter the electron transport chain at complexes I (NADH ubiquinone oxidoreductase) and II (Succinate dehydrogenase). They are then funneled through the electron carriers, Coenzyme Q and complex III (Ubiquinol cytochrome c oxidoreductase), until they reach complex IV (cytochrome c oxidase) where they are used to reduce molecular oxygen to water. This transfer of electrons is coupled to the extrusion of protons at complexes I, III and IV generating an electrochemical gradient across the mitochondrial membrane. Protons in the intermembrane space are then used to drive the synthesis of ATP at complex V (ATP synthase). C = cytochrome c. Dashed arrows indicates path of electrons.

Figure 2.

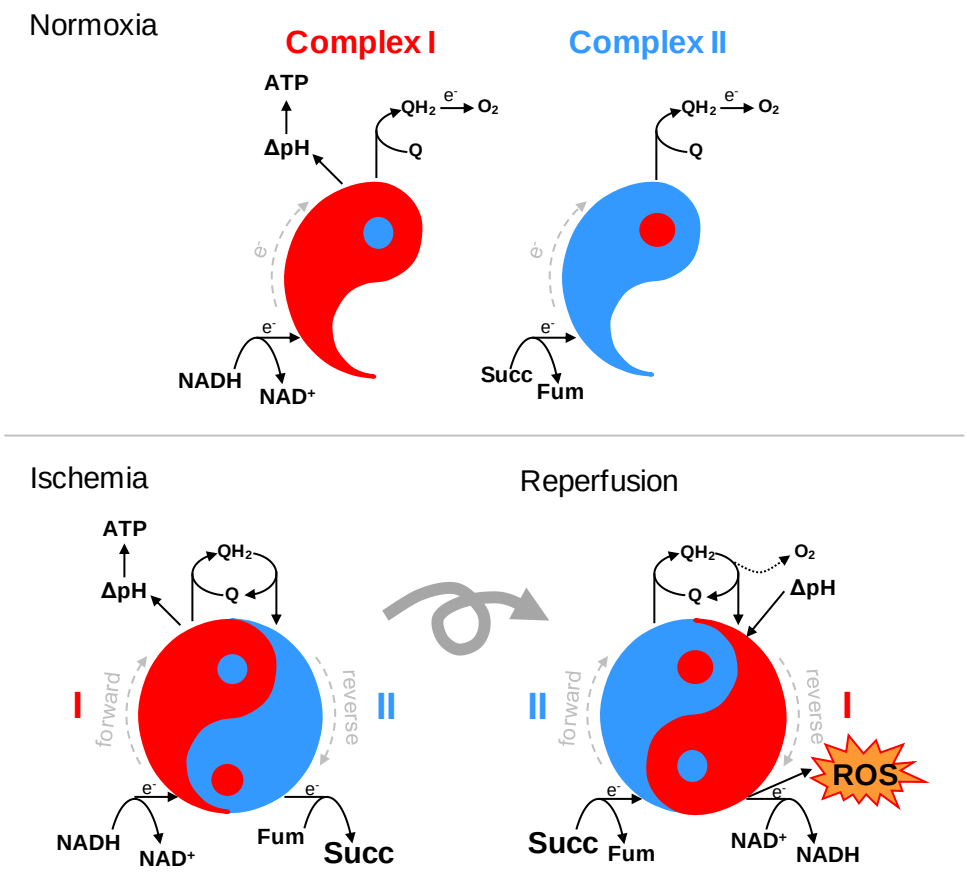

Figure 2. Respiratory Complex I & II "Yin-Yang" during ischemia & reperfusion. Under normoxic conditions, both complex I (red) and complex II (blue) work in the forward direction (dashed grey line indicates direction of electron flow), taking electrons from NADH and succinate respectively, and reducing ubiquinone (Q) to ubiquinol (QH_2). Electrons are eventually passed down the respiratory chain to O_2, and complex I pumps protons to generate a trans-membrane ΔpH. During ischemia, QH_2 generated by complex I working forward, is oxidized by complex II working in reverse. In this "Yin-Yang" formation, fumarate acts as an electron acceptor, resulting in accumulation of succinate. This process allows complex I to continue pumping protons during ischemia. At reperfusion, the rapid consumption of accumulated succinate generates too much QH_2 for the re-oxygenated terminal respiratory chain to handle (dotted line). Coupled with residual acidic pH from ischemia, this drives reverse electron transfer in complex I, resulting in the generation of significant amounts of ROS.