

MITOCHONDRIAL POTASSIUM CHANNELS AS PHARMACOLOGICAL TARGET FOR CARDIOPROTECTIVE DRUGS

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Abstract

Brief periods of ischemia are known to confer to the myocardium an increased resistance to the injury due to a later and more prolonged ischemic episode. This phenomenon, known as ischemic pre-conditioning (IPC), is ensured by different biological mechanisms. Although an exhaustive comprehension of them has not been reached yet, it is widely accepted that mitochondria are pivotally involved in controlling cell life and death, and thus in IPC. Among the several signaling pathways involved, as triggers and/or end-effectors, in the mitochondrial mechanisms of cardioprotection, an important role is played by the activation of potassium channels located in the mitochondrial inner membrane (mitoK) of cardiomyocytes. Presently, different types of mitoK channels have been recognized in the heart, such as ATP-sensitive (mitoK_{ATP}) and calcium-activated (mitoBK_{Ca} and mitoSK_{Ca}) potassium channels. Consistently, drugs modulating mitochondrial KCs, on one hand, have been employed as useful experimental tools for early basic studies on IPC. On the other hand, activators of mitochondrial KCs are promising and innovative therapeutic agents for limiting the myocardial injury due to ischemic episodes.

In this review, we report the experimental evidence supporting the role of mitoK channels in signaling pathways in the mechanisms of cardioprotection and an overview on the most important molecules acting as modulators of these channels, with their profiles of selectivity. Some innovative pharmaceutical strategies for mitochondriotropic drugs have been also reported. Finally, an appendix describing the main experimental approaches usually employed to study mitoK channels in isolated mitochondria or in intact cells, has been added.

Keywords

Ischemia-reperfusion, mitochondrial potassium channels, myocardial injury, potassium channel activators, cardioprotective drugs.

1- ISCHEMIA/REPERFUSION INJURY

Acute myocardial ischemia is one of the major causes of morbidity in western society and despite the recent advances in therapy, it remains a major cause of mortality. Indeed, the reduced coronary blood supply leads to cell death and loss of cardiomyocytes population, resulting in serious and often irreversible consequences on myocardial functionality [1]. The myocardial cell death during an ischemic episode is caused by necrosis, mostly due to the irreversible opening of the mitochondrial permeability transition pore (MPTP) and by the stimulation of the death receptor-induced necrosis, a process known as necroptosis [2-3]. Currently, the most effective strategy to reduce ischemic damage is an early reperfusion, but paradoxically the reperfusion in itself is responsible for an additional damage. Indeed, the global myocardial damage is referred to as ischemia-reperfusion (I/R) injury [4].

A *Ischemia-induced cell death*

During ischemia, the rate of glycolysis in the myocardial cell rises, in order to produce energy by mitochondrion-independent pathways; this causes a metabolic rise of lactic acid and acidification of the intracellular pH. Besides, the Na^+/K^+ ATPase is inhibited, because of the decline of ATP concentration and the intracellular $[\text{Na}^+]$ increase, then the Na^+/H^+ antiporter is inhibited, with further lowering of the pH. The $\text{Na}^+/\text{Ca}^{++}$ antiporter, that usually pumps Ca^{++} out of the cell and Na^+ into the cell, is inhibited or reversed, leading to an overload of Ca^{++} , responsible for hypercontracture, that contributes to the irreversible opening of MPTP [5] ATP is rapidly converted to ADP and AMP, which leak out of the cell and contribute to reduced cardiac performance. During the ischemia episode, the cytosol concentration of ROS exhibits a biphasic behaviour: in the first minutes, a small amount of ROS is produced; while after 20-25 minutes, a dramatically higher ROS concentration is formed. The depletion of

ATP, together with elevated intracellular $[Ca^{++}]$ and ROS, leads the cell to a gradual and irreversible decline of its integrity [5-7] (Figure 1A).

B Reperfusion-induced cell death

The beginning of the reperfusion is associated with a burst of ROS production, primarily formed by complex 1, 2 and complex 3 of the respiratory chain, but also by other mitochondrial oxidative enzymes, such as xanthine oxidase, NAPH oxidase, monoamine oxidase and aconitase [8-10]. The mitochondrial proteins are particularly susceptible to ROS-induced damage: ROS have direct effects on the respiration and play a critical role in the opening of the MPTP [5-7, 11, 12]. MPTP is a high conductance mega-channel, anchored between the mitochondrial outer and inner membrane. When it is assembled, it allows the connection between the cytoplasm and mitochondrial matrix [13]. It is formed by an arrangement of the voltage-dependent anion channel (VDAC), located in the outer mitochondrial membrane, with the adenine nucleotide transporter (ANT) and the proteic translocator (TSPO), located in the inner mitochondrial membrane, and cyclophilin D in the matrix [14-16].

It is thought that during ischemia the MPTP is closed, since the pore is powerfully inhibited by low pH (<7) [17]. Indeed the rapid energization of mitochondria at reperfusion leads to electrogenic uptake of Ca^{++} , previously accumulated into the cytosol during ischemia, that supports a “destructive” hypercontracture. This factor, together with the rise of ROS production and the recovery of neutral pH, promotes the opening of MPTP [18-20]. The opening of just a single pore in one mitochondrion is sufficient to cause its immediate depolarization [21], and then enlisting further MPTP opening, since the MPTP activation is triggered by depolarization. As a consequence of MPTP opening, all small molecular weight solutes (< 1.4 kDa) equilibrate across the inner membrane; in contrast, the largest molecules

(i.e. proteins) remain entrapped in the matrix, exerting an osmotic pressure that leads to the uptake of water and matrix swelling [22, 23]. Although the unfolding of the cristae allows the matrix to expand without rupture of the inter-membrane, the outer one breaks and leads to the release of pro-apoptotic proteins, confined in the inter-membrane space, such as cytochrome c [24-26]. There is an increasing evidence that the time of MPTP opening is closely correlated with the extent of damage. Indeed inhibitors of the pore opening (such as cyclosporine A) protect the heart from injury and many preconditioning strategies, aim at inhibiting opening of MPTP [27-30] (Figure 1B).

2- MECHANISMS OF CARDIOPROTECTION

A *Ischemic pre-conditioning (IPreC)*

Transient brief episodes of I/R (typically of 2-5 minutes) confer on myocardium an increased resistance against a subsequent prolonged severe episode of I/R. This phenomenon is known as ischemic pre-conditioning (IPreC) and is recognized as one of the most powerful endogenous cardioprotective mechanisms. IPreC was first described in dog hearts [31], and thereafter confirmed in many mammalian species, including humans [32-35].

In particular, two distinct phases of IPreC are recognized: an early one, the “classic IPreC”, that lasts for 3 h after the stimulus, and a delayed one, the “second window of IPreC”, starting about 24 h after the triggering stimulus and lasting for up to 3 days following the stimulus [32, 36-38].

Early IPreC is due to the involvement of already existing biomolecules acting as effectors; whereas the delayed one is mediated by newly synthesized cardioprotective proteins [39] (Figure 2).

B *Ischemic post-conditioning (IPostC)*

More recently, Zhao and colleagues demonstrated that brief intermittent cycles of coronary re-occlusion (typically 30 seconds) and reperfusion (30 seconds) during the first minute of reperfusion after a severe ischemic event, reduce the infarct size by about 40% in canine hearts [40]. As observed for the IPreC, also the IPostC has been confirmed in many other mammalian species [41-42]. Presently, the mechanisms involved in IPostC have not been fully understood, but reduction of ROS production, mitochondrial Ca⁺⁺ overload and inflammation have been observed [35,40, 43] (Figure 2).

C Remote ischemic pre- and post-conditioning (ReIPreC, ReIPostC)

Schmidt et al. demonstrated that brief intermittent cycles of limb ischemia afforded a significant protection during myocardial infarction, preserving the cardiac function and reducing the arrhythmias typical of reperfusion [44]. Similarly, Li et al. also observed that limb brief ischemia periods reduced the myocardial damage after an ischemic period by inhibiting oxidative stress [45]. Again, renal ischemia episodes, in the first minute of reperfusion, reduced the myocardial infarct size in rats [46].

These phenomena are called remote ischemic preconditioning (ReIPreC) and remote ischemic postconditioning (ReIPostC). They consist in brief episodes of I/R applied to an organ distant from the heart, before or after prolonged ischemia, and afford cardioprotection [46-48]. Although the molecular mechanisms accounting for ReIPreC and ReIPost are not well understood (most studies are largely observational), these phenomena have been clearly observed in many mammalian species, including rat, rabbit and pigs, and are likely to require the transfer of protective factors or signals through humoral and/or neural pathways, such as some kinases [49].

D Pharmacological pre-conditioning (Ph-PreC)

A clinical procedure of IPreC, anticipating a myocardial infarct, is almost impossible to be applied, since the infarct onset cannot be predicted. In contrast, a clinical cardioprotective strategy based on IPostC is conceptually feasible in patients with acute myocardial infarct, but it shows many difficulties, risks and disadvantages [47, 50]. The definition of the signaling pathways of IPreC and IPostC, paves the way to develop pharmacologic strategies, to mimic the protection with drugs able to trigger the same mitochondrial pathways (Figure 2). The IPreC, as well as IPostC which recruits analogous signaling pathways, is mediated by numerous endogenous factors, including adenosine [51], acetylcholine [52], bradykinin [53] and opioids [54-59] and gaseous molecules, such as NO [60-63] and H₂S [64].

Moreover, the IPreC and IPostC involve the activation of protein kinase C (PKC), isozymes which sometimes exert opposing roles in both normal signaling and disease states [65], and possibly other kinases, such as reperfusion injury salvage kinase (RISK), glycogen synthase kinase 3 beta (GSK3B), signaling transducer and activator of transcription 3 (STAT3) [63].

As regard the isoforms of PKC, the ϵ and δ isoforms play opposite roles in cardioprotection. In particular, PKC ϵ activation is cardioprotective, while PKC δ activation mediates the most part of the injury induced by myocardial ischemia [66]. Recent evidence shows that PKC ϵ activates the mitochondrial aldehyde dehydrogenase 2 (ALDH2), which removes products of lipid peroxidation [67] thus protecting the mitochondrial functions. Moreover, Baines et al. reported that the translocation of PKC ϵ can be considered as an additional mechanism for protection: the direct phosphorylation of the MPTP components inhibits the pore opening [38, 68, 69]. In contrast, PKC δ inhibition during the reperfusion phase is cardioprotective. Actually, the PKC δ activation triggers the mitochondrial pyruvate dehydrogenase kinase [70], thus inhibiting both pyruvate dehydrogenase and ATP regeneration. Moreover, PKC δ activation induces impaired perfusion of myocytes after the ischemic event, thus leading to further tissue injury [71]. However, the role of PKC for cardioprotection in mammalian is still

controversial. Heusch et al. reported that the PKC inhibitor staurosporine did not prevent IPreC in swine [72]. Moreover, after the first encouraging evidence of Mochly-Rosen that inhibition of PKC δ at the onset of reperfusion reduced the tissue injury, the clinical trial failed to show any significant benefit [73].

In addition to PKC isozymes, other different protein kinases have been proposed as mediators of cardioprotective pathway. For example, STAT3 has been recently identified in mitochondria and its expression is correlated with the reduction of myocardial injury by IPreC and IPostC. consistently, the deletion of STAT3 or its inhibition abrogated the cardioprotection mediated by conditioning strategies [74, 75].

Again, several types of potassium channels, present in the inner mitochondrial membranes and similar to those present in the plasma membrane of several cell types, have been suggested to be triggers and end-effectors in cardioprotection. Presently, ATP-sensitive [76] and calcium-activated [77-80] potassium channels have been recognized and has been proved to be involved in the regulation of mitochondrial volume, membrane potential, pH-regulation and apoptosis.

On the other hand, connexin 43, a transmembrane protein which allow direct communication between cytoplasm of adjacent cells, by the formation of gap junctions, has been described in mitochondria and demonstrated to participate in the IPreC [81]. Interestingly, mitochondrial connexin 43 contributes to mitochondrial potassium uptake, forming hemichannel-like structures or modulating existing ion transporters [81-83]. Noteworthy, connexin 43 is hypothesized to be involved in the cardioprotection induced by the K_{ATP} activator diazoxide, because, in connexin 43 deficient mice, diazoxide is devoid of beneficial effects [84].

3- MITOCHONDRIAL POTASSIUM CHANNELS INVOLVED IN CARDIOPROTECTION

The critical role of mitochondria and the involvement of mitochondrial pathways activated by IPreC or IPostC are crucial in the mechanism of cardioprotection. In this context, the mitochondrial potassium channels (mitoK) represent an attractive pharmacological target. In normal conditions, the mitochondrial inner membrane is almost impermeable to K^+ . Hence, when mitoK channels are closed, the entry of K^+ ions is negligible and easily buffered by a K^+/H^+ antiporter, that pumps K^+ out of the matrix; therefore the influence of such a K^+ leakage on the mitochondrial membrane potential is almost negligible, although it can regulate the matrix volume.

By contrast, in stress or ischemia conditions, the opening of mitoK channels causes a significant influx of K^+ ions, with diffusion of water and uptake of anions, resulting in matrix swelling. This effect ensures the preservation of low permeability of the outer membrane for nucleotides and then the creation of a favorable gradient for ATP synthesis and transfer to cytoplasm [85]. Then, the activation of mitoK channels controls the matrix volume, preserving a narrow intermembrane space, necessary to preserve an effective oxidative phosphorylation. Moreover, the opening of mitoK channels produces a mild depolarization of membrane potential, responsible for a reduced uptake of Ca^{++} into the mitochondrial matrix, preserving it from the Ca^{++} -overload and the subsequent MPTP opening [86].

Finally, the cardioprotection offered by mitoK channel activation can be also linked to a mild uncoupling of mitochondrial respiration [87] (Figure 3).

Effects of mitoK channel activators is summarized in table 1.

A *MitoK_{ATP} channels*

The first mitoK channel recognized as an effective target for Ph-PreC was the ATP-sensitive one (mitoK_{ATP}). K_{ATP}-openers, such as bimakalim and cromakalim, were reported to produce

cardioprotective effects at doses devoid of any influence on action potential duration, suggesting the existence of an intracellular site of action, independent of the sarcolemmal channels [88-91].

Later, the first direct evidence about a role of mitoK_{ATP} channels was the observation of the effect of the K_{ATP} opener diazoxide on bovine heart mitochondria, at a concentration clearly lower than that necessary for opening the sarcolemmal channel [92]. Although for several years there has been a debate on which type of K_{ATP} channel (sarcolemmal or mitochondrial) was involved in cardioprotection, presently, it is generally accepted that the mitochondria play a crucial role in the I/R event and the role of the mitoK_{ATP} channel is likely to be prevalent [93].

Indeed, numerous experimental reports in support of this hypothesis have been collected in the last years, demonstrating that the IPreC, and most recently even the IPostC, can be mimicked by several selective mitoK_{ATP} channel activators [94-98].

To date, no definitive molecular identity has been assigned to the mitoK_{ATP} channel; nevertheless, a resemblance with the sarcolemmal one has been proposed. In fact, mitoK_{ATP} is likely to be an hetero-octameric complex, containing K_{IR}6.1 or K_{IR}6.2 subunits and SUR2 sulphonylurea receptor subunits, while the SUR1 subunit seems to be excluded (for a more complete and authoritative examination of the molecular composition of mitoK_{ATP} channels: [99-102]). Recently, a mitochondrial-specific SUR2 variant (mitoSUR2), generated by an intraexonic splicing of the classical SUR2 subunit, has been described; noteworthy, in SUR2 knock-out mice, in which the mitoSUR2 form remained expressed, the IPreC strategy was still effective in reducing ischemic injury [103].

Moreover, the pharmacological cross-talk between the mitoK_{ATP} channel and mitochondrial respiratory complex II (succinate dehydrogenase) led to the hypothesis that complex II could be a further component of the mitochondrial channel [104]. Interestingly, a step forward in

understanding the molecular structure of the mitoK_{ATP} channel was achieved by the O'Rourke group: they demonstrated that the ROMK (renal outer medullary potassium) channel, normally expressed on the surface membrane in the kidney cells, is localized on the inner membrane of cardiac mitochondria and mediates ATP-sensitive K⁺ flux, giving protection against cell death stimuli. This observation led to speculation that mitoROMK may be a pore-forming subunit of the mitoK_{ATP} channel [105].

B MitoK_{ATP} activators

The first pioneering experiments probing the cardioprotective properties of K_{ATP} channel activators were carried out with cromakalim or its analogs. It is a benzopyran compound completely devoid of selectivity for the mitoK_{ATP} channels, thus the protective effects are endowed with vasodilatation and action potential duration shortening, due to the activation of the sarcolemmal K_{ATP} channels present in vascular smooth muscle cells and cardiomyocytes, respectively. The benzopyran nucleus represents the scaffold most widely subjected to chemical manipulation in order to develop novel mitoK_{ATP} activators [106].

Diazoxide, a prototype of the benzothiadiazine class, has been reported to open mitoK_{ATP} channels with a good, but not absolute, selectivity. At moderate doses it can activate mitoK_{ATP} channels, producing Ph-PreC, without showing non-selective effects. Nevertheless, at higher doses, an activation of sarcolemmal K_{ATP} channels cannot be excluded [107-108]. Although the role of ROS in the IPreC is controversial, Heusch and colleagues demonstrated that diazoxide conferred protection through the generation of free radicals [84, 109]. MitoK_{ATP}-independent mechanisms of diazoxide have also been hypothesized and a possible target is likely to be the mitochondrial succinate dehydrogenase [110-112]. Indeed, Wojkovich and his colleagues proved that the complex II is an important regulator or component of the mitoK_{ATP} channel, since diazoxide is able to activate the channel, but also to inhibit the complex II;

conversely, a complex II inhibitor atpenin A5, is able to open at lower concentration the mitoK_{ATP} channel [113-114].

Unfortunately, diazoxide is also responsible for undesired vasodilator and hyperglycaemic effects [95, 115-116].

Attempting to ameliorate the selectivity versus the mitoK_{ATP} channel, the Bristol Mayer Squibb Company has developed original hybrids, conjugating the benzopyran moiety of cromakalim with the cyanoguanidine nucleus (another important scaffold of non-selective K_{ATP} openers, such as pinacidil) [106, 117].

This research led to two interesting compounds: BMS 180448, endowed with a good cardioprotective potency, but still conserving a poor vasorelaxing potency and BMS191095, a 4-N aryl-substituted benzopyran, were synthesized. The latter molecule resulted about 30-fold more selective than BMS 180448 and its pharmacological effect was antagonized by 5-hydroxy decanoic acid (5-HD), a selective mitoK_{ATP} blocker. Nevertheless, BMS 191095 is not suitable for clinical use, because of its neuronal toxicity; it remains only a useful tool for research purposes [118]. Afterwards, Lee and his colleagues developed new molecules based on a benzopyran scaffold bearing a 2'-carboxyalkyl-indole or 2'-carboxyalkyl-indoline nucleus in 4-position. In particular two 2-ethyl-esters showed the best cardioprotective activity in vivo and in vitro, and a weak vasorelaxing potency. The cardioprotective effect of these novel compounds was completely blocked by 5-HD, suggesting the involvement of the mitoK_{ATP} channels [119]. Moreover, the benzopyran-yl-indole analog KR-31466 reduces hypoxic injury in heart-derived H9c2 cells through mitoK_{ATP} opening. Jung et al. suggested that this protection from hypoxia-induced death could also involve PKC activation [120].

More recently, a novel series of spiro-morpholine and spiro-morpholone benzopyran derivatives has been projected and synthesized by us [95, 121-123]. The insertion of spiro-cyclic substitution on the carbon 4 of the benzopyran group had the objective to confer a

structural rigidity in this molecular portion, in order to improve the selectivity vs the mitoK_{ATP} channel.

Some compounds of this series exhibited a good cardioprotective profile in ex vivo models of I/R and modest effects on vascular smooth muscle. In particular, the best of the series, the N-acetyl spiro-morpholone derivative (F163) has been submitted to further studies. The resolution of the racemic mixture, according to the results previously obtained with BMS 180448, showed the enantioselectivity of the cardioprotective profile; in fact only the levorotatory enantiomer was effective [124]. An in depth pharmacological investigation confirmed the cardioprotection on cardiomyoblasts culture submitted to anoxia/reperfusion and in vivo on acute myocardial infarct; moreover the mitochondrial target of F163 has been demonstrated [95]. Actually, it was observed that the novel compound was able to produce all the typical effects of a mitoK channel activation: mild depolarization, reduction of Ca⁺⁺-uptake, increase of Ca⁺⁺-release and mild mitochondrial swelling, reversed by selective mitoK_{ATP} blockers. According to the literature, a close correlation between drug-induced depolarization and Ca⁺⁺-release from the matrix of Ca⁺⁺-preloaded mitochondria was also reported, leading to the observation that a small depolarization is sufficient to greatly affect the movement of Ca⁺⁺. This study suggested that the novel derivative could represent an interesting prototype for the development of innovative mitoK_{ATP} openers, that are more potent than diazoxide and devoid of significant influences on blood pressure and glucose metabolism [95] (in figure 4 are shown the chemical structures of K_{ATP} activators).

Presently, no specific study attempted to identify the binding site of K_{ATP} activators on the mitoK_{ATP} channels; however, in analogy with the sarcolemmal channels, the interaction with the SUR subunit seems to be more probable hypothesis.

C Mitochondrial calcium-sensitive potassium channels

C.1 MitoBK_{Ca} channels and their activators

In 2002, O'Rourke's laboratory for the first time found the presence of a large conductance calcium-activated potassium channel (BK_{Ca}, or K_{Ca}1.1) on the guinea-pig cardiac inner membrane of mitochondria (mitoBK_{Ca}) [79], previously described only on mitochondria of glioma cells without assigning a specific function [77]. In this work, they employed patch-clamp mitoplasts and identified the K⁺ current across this channel as a component of background K⁺ conductance. Moreover, they identified a role for the mitoBK_{Ca} channel in the protection against ischemic injury. Indeed, the BK_{Ca} activator, NS1619, decreased the infarct size following an I/R period, and this effect was antagonized by the BK_{Ca} blocker paxilline [79, 104]. Other reports have also confirmed that the pharmacological opening of mitoBK_{Ca} channels mimicked both early and delayed preconditioning [125-127], as well as postconditioning [94].

Noteworthy, cardiopreventive effects of NS1619 seemed to be also mediated by an anti-inflammatory activity that prevented leukocyte-endothelial cells adhesion and preserved mitochondrial functionality [128-130].

However, micromolar concentrations of NS1619 cause also anomalous effects on mitochondria, such as decreased respiratory control, insensitivity to selective BK_{Ca} blockers, and a deep membrane potential drop even in the absence of K⁺ [131-132]. Such a profile of NS1619, similar to diazoxide, is due to off-target effects on mitochondria, including inhibition of the respiratory chain and uncoupling [133]. Notably, at higher concentrations NS1619 can also inhibit L-type Ca⁺⁺-channels, calcium-activated Cl⁻ currents, voltage-gated calcium channels and K⁺ and Na⁺ channels; these aspecific effects complicate the interpretation of the role of mitoBK_{Ca} channels in cardioprotection [134-137].

Another novel BK_{Ca} activator, chemically unrelated to, but more potent and specific than NS1619, is NS11021 [138]. It exerts protective effects through activation of BK_{Ca} channels; these effects were completely inhibited by paxilline. Nanomolar concentrations of NS11021 improved the bioenergetic performance of heart mitochondria, through enhanced K⁺-uptake and mild swelling, without large changes in mitochondrial membrane potential ($\Delta\Psi$). Interestingly, the improvement of mitochondrial respiration observed with NS11021 by Aon et al. was due to a decrease in state 4 respiration (related to the phase in which all of the ADP is converted to ATP), while the state 3 respiration (corresponding to the phase in which ADP is converting to ATP) was unchanged. Such an unexplained behavior of NS11021 is different from mitoK_{ATP} openers, which dose-dependently improve respiration through an increase of state 3 [139-140].

Besides the most extensively studied NS-series, the more potent compound CGS7184 (ethyl 1-[(4-chlorophenyl)-amino]oxo]-2-hydroxy-6-trifluoromethyl-1H-indole-3-carboxylate) has been developed [141]. CGS7184 reduced the ROS production in mitochondria isolated from rat brain [142], and depolarized the $\Delta\Psi$ in the EAhy926 endothelial cell line [143]. These effects, that could be predictive of protective properties, seem to be related to modulation of intracellular Ca⁺⁺ homeostasis, probably by interaction with the sarcoplasmic reticulum [144]. The molecular composition of mitoBK_{Ca} channels is yet unknown; although an auxiliary beta1 subunit, necessary for the IPreC and identical to the sarcolemmal one, was found [145-146]; however some splice variants at the mitochondrial level cannot be excluded. Presently, it is thought that the mitochondrial and sarcolemmal structures are conserved, and then the mitoBK_{Ca} is likely to be composed of four alpha subunits, forming the pore, and four auxiliary beta-subunits, which modulate calcium sensitivity and activity [147].

Very recently, the Toro group unequivocally demonstrated the existence of the KCNMA1 (Slo1) gene at the mitochondrial level, encoding for BK_{Ca} channel. Their hypothesis was

supported with experiments on *Kcnma1* knockout mice, in which NS1619 failed to produce cardioprotection [148].

Notably, the combined application of sub-maximally effective concentrations of two different mitoK activators NS1619 and diazoxide on cardiac mitochondria, produced additive effects, reducing flavoprotein oxidation and ouabain-induced mitochondrial Ca^{++} overload; thus contributing to cardioprotection [149] (in figure 4 are shown the chemical structures of BK_{Ca} activators).

C.2 MitoIK_{Ca} channels

Besides the mito BK_{Ca} channels, an intermediate conductance calcium-sensitive potassium channel (IK_{Ca} , or $\text{K}_{\text{Ca}3.1}$) has been detected in purified mitochondria of human colon carcinoma cells [99, 150], of HeLa cells and embryonic fibroblasts [151]. Its molecular weight is the same of IK_{Ca} channel localized on plasma membrane, suggesting equivalent biophysical and pharmacological properties [151]. The physiological role of mito IK_{Ca} has not been studied in detail so far, but TRAM-34 (an IK_{Ca} inhibitor) induced hyperpolarization of the mitochondrial membrane, in agreement with the typical profile of mitochondrial potassium channel activators [151]. At the best of our knowledge, mito IK_{Ca} channels have not been presently recognized in the heart.

C.3 MitoSK_{Ca} channels

Very recently, a small conductance calcium-sensitive potassium (SK_{Ca} , or $\text{K}_{\text{Ca}2}$) channel has been identified and localized thanks to immune-electron microscopy and purified by isoelectric focusing on the inner membrane of guinea-pig cardiac mitochondria and in mitochondria of neuronal cells [152]. The authors furnished convincing evidence of the presence of mito SK_{Ca} channels, and also demonstrated that their activation triggers the Ph-

PreC and then confers cardioprotection, as shown by metabolic and functional improvement recorded during the reperfusion on isolated and perfused guinea-pig hearts. Finally, the activation of mitoSK_{Ca} channels appears to converge, similarly to mitoBK_{Ca} and mitoK_{ATP} channel opening, on the control of intra-mitochondrial ROS generation [80, 125, 133].

Within the compounds tested as activators of mitoSK_{Ca}, DCEBIO was shown to induce cardioprotection which was antagonized by both NS8593 [153] an antagonist of SK_{Ca} isoforms and TBAP, a scavenger of peroxynitrite (SOD mimetic), thus indicating that the mitoSK_{Ca} channel opening is mediated by SOD [80] (in figure 4 is shown the SK_{Ca} activator).

D MitoKv channels

In 2005, Szabò and colleagues found the presence of voltage-gated 1.3 potassium (Kv1.3) channels on the inner membrane of mitochondria in T lymphocytes [154]. Later, Kv1.3 channels were also reported in the mitochondria of macrophages [155] and hippocampal neurons [156].

Although the Kv1.3 channels described in mitochondria of hippocampal neurons displayed a different conductance with respect to the Kv1.3 channels on lymphocytes, both channels are sensible to margatoxin. Interestingly, a hyperpolarization of mitochondrial membrane potential could be recorded upon inhibition of mitoKv1.3, indicating that in energized mitochondria these channels are normally active [154], and their inactivation is important in the initiation of apoptosis [157].

The Zoratti/Szabò group further reported that J774 macrophages express Kv1.3 and Kv1.5 channels in their mitochondria and that inhibition of both channels with specific blockers could efficiently induce apoptosis in this macrophage cell line. Thus, these results indicate that the mechanism proposed for Kv1.3 can be extended to other Kv channels in

macrophages, important players in the immune system [158-159]. Noteworthy, Leanza et al. have recently shown that the inhibition of mitoKv1.3 induces a drastic effect on tumor cell apoptosis by means a Bax/Bak-independent pathway [160-161].

At the best of our knowledge, mitoIK_{Ca} channels have not been presently recognized in the heart.

E MitoTASK-3 channels

Recently, TASK-3 (KCNK9), one of the twin-pore domain potassium channels, was described in mitochondria of melanoma, keratocyte cells [162] and embryonic rat hippocampus [163], while its functional properties remain to be largely investigated [164]. In 2014, the Szewczyk group demonstrated that this channel has a protective role in human keratinocytes after UVB radiation exposure [165]. At the best of our knowledge, mitoTASK-3 channels have not been presently recognized in the heart.

F “Dirty” drugs able to activate mitoK_{ATP} and mitoBK_{Ca} channels.

Levosimendan, a cardiovascular drug used to treat acute and decompensated heart failure, owes its action to a calcium sensitization of the contractile proteins and to a vasorelaxing effect mediated by the opening of sarcolemmal K_{ATP} channels [166-167]. Recently, levosimendan has been observed to activate also the mitoK_{ATP} channels [168-169] and has been demonstrated to protect myocardium against the I/R injury [170].

Moreover, it has been speculated that the cardioprotective effects of numerous polyphenols (such as theaflavin and epigallocatechin) are due to an activation of mitoK_{ATP} channels [171].

Volatile anesthetics activate some of the same pathways that lead to protection through IPreC or IPostC. Zaugg et al. verified that exposing cardiomyocytes to the volatile anesthetics, sevoflurane and isoflurane, before myocardial ischemia, the damage decreased in a dose-dependent manner [172]. These effects were antagonized by the mitoK_{ATP} channel blocker 5-HD, demonstrating that they prime the mitoK_{ATP} channels [173-175].

In addition, Stumpner et al. reported the involvement of the BK_{Ca} channel in the desflurane-induced post-conditioning in mice subjected to a coronary artery occlusion [176].

Other molecules, not described as BK_{Ca} openers, belonging to different therapeutic class, such as phosphodiesterase inhibitors (cilostazol and sildenafil), can mimic the Ph-PreC by an activation of mitoBK_{Ca} [177, 146].

Very recently it has been demonstrated that naringenin, a flavanone abundant in the Citrus genus, possesses cardioprotective activity by opening the mitoBK_{Ca} channels. Indeed, naringenin treatment before the onset of in vivo acute infarct or a global ischemia halved I/R injury sizes. Moreover, on isolated cardiac mitochondria, naringenin was able to produce all the typical effects of the mitoK channel openings, and the involvement of mitoBK_{Ca} was suggested by the inhibitory effect of selective blockers [178]. Similarly, Gao et al., published a work on the cardioprotective effect of the isoflavone puerarin. They observed the involvement of mitoBK_{Ca} channels, since the pretreatment with paxilline prevented its beneficial effect [179] (in figure 5 are shown the chemical structures of the dirty drugs).

G Innovative mito-delivered drugs

Ideal mitoK channel openers should have the ability to easily penetrate into the cell and be effectively delivered to their mitochondrial target. The lack of this pharmacokinetic property is a serious limitation for the clinical application of mitoK channel openers. Therefore a

timely and challenging issue is to obtain anti-ischemic drugs highly selective for the mitoK channels and able to be selectively delivered into mitochondria (in figure 6 are shown the chemical structures of the mito-delivered drugs). For this purpose, the most widely used synthetic strategy is the combination in a single molecule of the pharmacophore moiety and a “mitochondria-addressed” cation, such as tetraphenyl phosphonium (TPP⁺), throughout an appropriate aliphatic linker. Generally, the length of the spacer influences the degree of hydrophobicity of such mitochondriotropic agents and consequently it could affect their efficacy both in *in vitro* and *in vivo* models [180-181]. These compounds are able to rapidly and extensively accumulate into mitochondria, driven by the mitochondrial membrane potential (about -180 mV)[182-183]. The main advantages in the use of lipophilic cations are their low reactivity and poor interaction with cell components. From a medicinal chemistry point of view, an example of synthetic pathway to obtain mitochondriotropic compounds is depicted in figure 7. In this instance, in order to deliver resveratrol into mitochondria, this polyphenol has been combined with TPP⁺ [184] (Figure 7A). In particular, resveratrol has been submitted to an O-alkylation by the use of an appropriate bis-halide-alkyl chain and then converted to the desired TPP⁺ derivative by means of two consecutive nucleophilic substitution steps: Cl⁻ → I⁻ → TPP⁺I⁻. A most recent paper [185] reports the synthesis and the pharmacological profile of SkBQ (10-(*p*-benzoquinonyl)decyltriphenylphosphonium bromide). As regards the synthetic pathway, also in this case the *p*-benzoquinone was alkylated with the appropriate alkylcarboxylic acid by the use of a radical substitution reaction with simultaneous decarboxylation catalyzed by silver nitrate in the presence of ammonium persulfate. The product was then converted to the desired TPP⁺ derivative by means of a nucleophilic substitution (Br⁻ → TPP⁺I⁻) (figure 7B). The above examples of synthetic pathways can be usefully applied to a plethora of different compounds in order to target them to mitochondrion. Many TPP⁺-based antioxidants have been tested for their

selectivity to mitochondria, including MitoVitE, MitoQ and MitoL. To date, a TPP⁺ derivative of MitoQ, an ubiquinol antioxidant, has received great attention, since it is endowed with cardioprotective effects in the I/R injury model [186-188]. In a recent study Mito-Tempol (a piperidine nitroxide conjugated to the TPP⁺) exhibited cardioprotective effects in an in vivo rat model [189].

Besides the lipophilic cations, some peptides can also be used to direct molecules to mitochondria. Particularly the Szeto-Schiller (SS) peptides are able to selectively bind to the inner membrane, independently of potential. This feature is an advantage, because they can penetrate even into the diseased organelles with reduced potential [190]. An important difference between lipophilic cations and the SS-peptides is that the latter have an intrinsic activity on mitochondria. In particular, SS-02 and SS-31 were able to scavenge hydrogen peroxide, hydroxyl radical, and peroxynitrite in vitro [191] and the observed decrease of myocardial I/R injury in ex vivo and in vivo studies was attributed to their ability to promote mitochondrial respiration and ATP synthesis, reduce ROS generation, and inhibit mitochondrial swelling [192]

Noteworthy, new penetrating cations have been very recently developed. They are rhodamine-19, berberine and palmatine conjugated through aliphatic linkers with plastoquinone (SKQR1, SKQBerb, SKQPalm, respectively), endowed with antioxidant effects; they have been shown to penetrate into mitochondrial matrix, both in isolated mitochondria and in living cells in culture [193].

4- LOSS OF EFFICACY OF MITOCHONDRIAL POTASSIUM CHANNEL ACTIVATORS IN AGEING

Presently, the number of people over the age of 65 is growing, especially in western society. The incidence and the prevalence of cardiovascular diseases, such as coronary artery disease, hypertension and acute myocardial infarct, increases proportionally with age. At the same time the IPreC and IPostC self-defence mechanisms reduce their efficacy and the vulnerability of myocardium increases with ageing [194]. Therefore an amplification of the damage is observed when aged hearts are exposed to various kinds of stress. The loss of intrinsic myocardial tolerance to ischemia in mouse myocardium begins during middle-age (12 months) and becomes more evident with ageing (18 months and 24-28 months). Similar results have been observed in rats (6 vs 24 months) [195-198]. The fundamental processes that lead to cardiac ageing and loss of myocardial resistance are not yet understood, although several mitochondrial defects have been detected, including a reduced activation of STAT3 observed in old mice [199] or an impaired connexin 43 translocation in the mitochondria of aged heart, that may contribute to the loss of efficacy of cardioprotective strategies [200] It is thought that the mitochondrial dysfunction in electron transport or oxidative phosphorylation, typical of ageing, is closely implicated in the increased susceptibility to ischemic injury. These age-related mitochondrial defects are reflected by partial depolarization of membrane potential and greater probability of MPTP opening [11, 201].

In this respect, the mitoK_{ATP} channel activation, that is generally considered an effector in the protection pathway, can lead to ineffective cardioprotection in ageing hearts [202]. Several observations suggested that cardioprotective signaling pathways, acting through mitoK_{ATP} channels, are compromised with ageing; although it is unclear whether the attenuated cardiac mitoK_{ATP} channel function is due to changes in channel density, responsiveness to stress stimuli or a defective respiratory chain [203-204]. Similar data have been also obtained when mitoBK_{Ca} channel openers were used on aged hearts. In aged rat heart mitochondria, Heinen and his colleagues have observed that the opening of mitoBK_{Ca} channels by NS1619 was

ineffective on respiratory parameters and then was unable to influence the respiratory efficacy, suggesting the possibility of a decreased sensitivity of mitoBK_{Ca} channels due to ageing [205].

This result is ageing-dependent [206-207], but also gender-dependent. In fact, the infarct-limiting efficacy of diazoxide is less evident in aged male hearts, and entirely absent in aged female hearts, suggesting a more severe defective function of mitoK_{ATP} channels in aged female hearts [208]. Such evidence leads to hypothesize the implication of estrogens in the ageing; in fact, these hormones may stimulate directly (acting on specific receptors localized in the organelle) and indirectly (regulating the mitochondrial transcription) the mitochondrial function [209]. Moreover, a recent paper highlighted an altered expression of six proteins in cardiac mitochondria of aged female rats [210].

Since human life expectancy increases due to effective treatment of cardiovascular and other diseases, there is a stringent need for the development of strategies designed to preserve the efficiency of cardioprotective mechanisms also in aged hearts or to slow ageing.

Although numerous drugs produce Ph-PreC in experimental models, none has been translated to clinical use, with the consequence that currently there is no FDA approved drug for reducing I/R-induced myocardial injury [211].

Clearly, the blockage or the reversal of the dysfunction of mitochondrial ion channels may improve the function of the organelle, and may also be beneficial in slowing down the process of mitochondria ageing, and the related pathologies .

5 CONCLUSIONS

Mitochondria play a unique and crucial role in the cardiac protection against I/R injury, then emerging therapeutic approaches are focused on the development of new molecules acting on specific mitochondrial sites. In this respect, the modulation of the flow of different ion species

across the mitochondrial membrane is considered as a greatly promising pharmacological approach. Since the accumulation of Ca^{++} into the matrix is a crucial step, in the dramatic chain of events leading to I/R-induced cell death, new intriguing strategies are addressed at reducing Ca^{++} accumulation by direct pharmacological inhibition of the Ca^{++} uniporter and of the MPTP [212-215]. Together with these newest pharmacological targets, mitochondrial potassium channels represent a reliable and well tried field of research. Indeed, extensive research has been performed in the last years, in order to highlight the exact role of these mitochondrial ion channels and innovative drugs have been developed in order to mimic conditioning phenomena. Nevertheless, to date, there are still many dark sides: 1) the exact molecular composition of many mitochondrial channels remains elusive; 2) the mitochondrial pathways triggered by the activation of these channels have not been clearly understood [216]; 3) possible changes in expression/activity of mitochondrial potassium channel in elderly patients should be better clarified; 4) similarity between sarcolemmal and mitochondrial potassium channels may compromise the development of selective drugs.

Many difficulties can be correlated, at least in part, with the internal localization of these channels, hardly accessible with the usual experimental techniques, and data should be carefully interpreted.

Below we have inserted an appendix in which we attempted to describe the most used experimental approaches aimed at studying mitochondrial potassium channels and their physio-pathological roles, on intact cell or in isolated organelles.

6- APPENDIX

Techniques used to characterize mitochondrial potassium channels

Approaches on isolated mitochondria are better for mechanistic studies, in order to highlight a specific mitochondrial target. The investigation is clearer, lacking the interference with cytosolic factors; however, sound protocols of isolation have to be carry out, in order to avoid damage of mitochondria [217], since they may be unavoidably aggregated or damaged by unreliable procedures.

Conversely, working on intact cells, shows the advantage to leave the organelles in their cellular environment, where the interactions with the cell are preserved, then obtaining results with a greater physiological relevance, without artifacts due to mitochondria isolation [218]. Nevertheless, the results can be more complex and difficult to understand, mostly if a mitochondrial channel has to be investigated.

However, the main obstacle in studying the mitoK channels is the availability of reliable assays. As above described, the opening of a mitoK channel is responsible for cardioprotection through a mild depolarization and consequent reduction of Ca^{++} -uptake and increase of Ca^{++} -release, thus reducing the probability of MPTP opening and apoptotic death. Therefore the possible strategies of study are two: to directly record the potassium movements, or to evaluate the mitochondrial downstream events consequent to the K^+ movements.

A Direct measurements of potassium ions movements

In order to achieve a direct measurement of the mitoK current the work on isolated mitochondria is fundamental, since undesired recording of sarcolemmal K^+ currents cannot be excluded in intact cells. An important contribution to understand the pharmacological properties of the mitoK channels comes from the use of electrophysiological techniques, where mitochondria are patched by micro-electrode and the current of the single potassium channel can be recorded. For this purpose, two methods can be used: voltage-clamp on planar

lipid bilayer and patch-clamp on mitochondria or mitoplasts [219]. The first one is based on the incorporation of purified inner or outer mitochondrial membrane fragments, containing the mitochondrial channel of interest, with liposome to form bilayer vesicles. This method allows the study of channels, which are not easily accessible for the patch-clamp technique [220].

On the other hand, the patch-clamp method on intact mitochondria has the advantage of holding the channels in their natural environment, because the isolation process for the bilayer method may be harmful for the channel. Disadvantages of the patch-clamp technique are: a) mitochondria are in close contact with membranes of the endoplasmic reticulum and it might be difficult to completely remove these membranes [221]; b) some channels might not be accessible for the glass pipette; thus, the patch clamp technique might fail to detect some important ion channel currents [222].

Even mitoplasts, prepared from the mitochondrial fraction by a swelling procedure or with French press technique [223] have been successfully employed for electrophysiological measurements of K^+ channel currents. In particular, Inoue et al., used this technique to demonstrate for the first time the activity of mitoK_{ATP} channels [76].

The major advantage of this technique is the possibility to work only with the K^+ channel of the inner membrane; in fact it is well known that most preparations of isolated mitochondria can be contaminated with plasma membranes, unless further purified by density gradient centrifugation and even then it is difficult to achieve complete removal of contaminating membranes [224].

In isolated mitochondria, potassium movements can be also directly measured using K^+ -sensitive mini-electrodes or fluorescent indicators.

K^+ -sensitive mini-electrodes are available, however they are not satisfactorily sensitive and reliable [225]. Instead, potassium binding fluorescence indicators are more sensitive and

allow the recording of changes of potassium ions in the mitochondrial matrix, as reported by Costa et al., 2006 [80, 133, 226].

Alternatively, fluorescent probes sensitive to thallium (Tl^+), a potassium-mimetic cation, are either commercially available or can be easily homemade. The ionic radii of Tl^+ and K^+ are similar, and thus heavy metal is widely and originally used to study sarcolemmal transmembrane K^+ flow. Such an experimental approach has been recently applied to evaluate mitoK currents and published by Wojtovich and his colleagues, and used also by us [113, 178].

B Indirect measurements of potassium ions movements

Most studies on the mitoK channels to date use indirect approaches on isolated mitochondria or on intact cells, by measuring downstream effects the mitochondrial K^+ uptake, such as changes in respiration, matrix alkalinization, flavoprotein fluorescence, and swelling-induced light scatter [227]. Nevertheless, the reliability of these methods is limited by the ability of other mitochondrial phenomena (e.g, electron transport chain activity, volume changes, membrane potential) to interfere with the measured parameters.

As regards flavoproteins, they are able to emit autofluorescence in the oxidated, but not reduced state [228], hence this can reflect the electronic trafficking of mitochondria and can be used to monitor the metabolic state of cells. Indeed, the autofluorescence of flavoproteins in intact cardiac myocytes is used as an indirect indicator of mitochondrial K^+ channel activity. Marban and colleagues reported that diazoxide caused an oxidation of mitochondrial flavoproteins, reversed by 5-HD. Similar effects have been reported in guinea-pig myocytes using NS1619. However, this experimental approach has many limitations, and other researchers have failed to reproduce these results, speculating that non-specific actions of diazoxide or NS1619 could influence the assay [229-230]. In the case of diazoxide, the

increase in flavoprotein fluorescence can be attributed to the inhibition of succinate dehydrogenase, protonophoric uncoupling, or even transient opening of the MPTP.

The swelling assay is based on the fact that the K^+ uptake into isolated mitochondria is followed by osmotically-obliged water, leading to mild swelling, thus the ion movements lead to changes in matrix volume, that can be measured at a wavelength of 520 nm. In particular, the swelling causes a decrease of refractive index. This assay has been criticized as irreproducible by some laboratories, because the mitochondrial isolation timing appears to be a critical factor.

Another approach used to investigate the activity of mitoK channels either in cardiac myocytes and in isolated mitochondria, is based on the recording of the depolarization of the mitochondrial membrane potential. Lipophilic cationic compounds are often used for the determination of $\Delta\Psi$, in fact these compounds permeate the mitochondrial membranes and distribute in accordance with the Nernst equation; thus the accumulation in the mitochondrial membrane matrix is driven by $\Delta\Psi$.

These lipophilic ions include fluorescent, radiolabeled and unlabeled probes.

As regards the unlabeled probes, they can be determined by ion-selective electrodes, that allow the continuous monitoring of changes in mitochondrial membrane potential. The lipophilic cation most used is the tetraphenylphosphonium (TPP^+), with its selective electrode [95, 178, 231, 232].

Direct measurements of membrane potential can be also carried out with fluorescent probes, monochromatic lipophilic cations, such as dihexyloxacarbocyanine ($DiOC_6(3)$) and rhodamines, or JC-1 (5,5',6,6'-tetrachloro-1,1',3,3'-tetramethylbenzimidazolylcarbocyanine iodide), whose emission spectrum shifts from red, when it forms into mitochondria J-aggregations, to a green emission reflecting the monomeric form of JC-1, due to the exit from mitochondria after membrane depolarization. The ratio of this green/red fluorescence is

independent of mitochondrial shape, density, or size, but is influenced only by the membrane potential. Fluorescence probes are largely used on intact cells, because if it has at its disposal isolated mitochondria, easier techniques are available. The choice of the probe is related largely to their equilibration characteristics and also to their toxicity versus the electron transport chain.

A net K^+ uptake results necessarily in matrix alkalinization, because overall transport must be electroneutral, then the K^+ influx will be balanced exactly by the electrogenic H^+ efflux driven by the electron transport. In other words, the K^+ for H^+ exchange would increase matrix pH by an amount determined by the buffering power of the matrix. Costa et al. [233] reported that the increase in matrix K^+ , mediated either by $mitoK_{ATP}$ or by potassium ionophor valinomycin, was accompanied by matrix alkalinization. This effect accounts for the role of $mitoK_{ATP}$ in cardioprotection and can be measured by means of pH-sensitive fluorescence probes (such as 2',7'-biscarboxyethyl-5(6)-carboxyfluorescein) [133, 234].

Finally, the $mitoK$ opening influences calcium movements across the mitochondrial membrane. Changes of Ca^{++} movements can be recorded by means of specific calcium-sensitive mini-electrodes. Using this approach, Holmuhamedov and colleagues observed that diazoxide and pinacidil reduced mitochondrial Ca^{++} uptake, in agreement with the evidence that the Ca^{++} -accumulation through the Ca^{++} uniporter is driven by the difference in membrane potential across the inner mitochondrial membrane. Conversely, this approach allowed to demonstrate also that $mitoK$ channel openers can promote Ca^{++} discharge from Ca^{++} pre-loaded mitochondria, phenomenon at the basis of cardiac protection [226, 235].

The primary mitochondrial Ca^{++} release pathway includes the MPTP, which can be activated by mitochondrial membrane depolarization and swelling [236-238]. Activation of $mitoK_{ATP}$ channels has been associated with mitochondrial membrane depolarization and can prime

swelling [107, 231], transiently activating the MPTP, and thus causing Ca^{++} discharge from preloaded mitochondria.

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9- LEGENDS

Figure 1

A) Schematic representation of events occurring during ischemic period.

The Na^+/K^+ ATPase is inhibited (1) because of the decline of ATP concentrations, leading to Na^+ accumulation and membrane depolarization, which triggers the opening of voltage gated Ca^{++} channels (2). Because of the high cytosol concentration of Na^+ , the $\text{Na}^+/\text{Ca}^{++}$ antiporter, that usually pumps Ca^{++} out of the cell and Na^+ into the cell, is inhibited or reversed, leading to further overload of Ca^{++} (3). The mitochondrial matrix massively accumulates Ca^{++} (4). During ischemia, the rate of glycolysis rises causing a metabolic production of lactic acid. The high intracellular concentration of Na^+ inhibits the Na^+/H^+ antiporter (5), with consequent lowering of the cytosol pH. Although the Ca^{++} accumulation in the matrix would be a triggering stimulus for MPTP opening, the acidic pH inhibits the activation of MPTP (6).

B) Schematic representation of events occurring during reperfusion.

During reperfusion, the reactivation of aerobic metabolism restores the levels of ATP. The Na^+/K^+ ATPase activity is restored (1), leading to the normalization of the cytosol concentration of electrolytes (2) and H^+ (3). In conditions of physiological pH, the high levels of matrix Ca^{++} triggers the opening of MPTP (4), with consequent activation of pro-apoptotic pathways.

Figure 2

Schematic representation of I/R event, IPreC, IPostC and PhPreC protocols.

Figure 3

Schematic description of one of the different mechanisms which links the activation of mitoK channels to the control of mitochondrial calcium movements and modulation of the MPTP. The activation of mitoK channels causes inward flow of potassium ions, weak membrane

depolarization and reduced driving force for calcium accumulation in the matrix, limiting the formation of MPTP and its opening. This mechanism can, at least in part, reduce the release of mitochondrial pro-apoptotic factors during reperfusion and thus preserve mitochondrial membrane integrity.

Figure 4

Chemical structures of non-selective K_{ATP} channel openers (cromakalim, diazoxide and pinacidil), selective mito- K_{ATP} activators (BMS derivatives, F163, and KR-31466), BK_{Ca} openers (NS1619, CGS7184, NS11021) and SK activator (DCEBIO).

Figure 5

Chemical structures of “Dirty” drugs able to activate mito K_{ATP} and mito BK_{Ca} channels.

Figure 6

Chemical structures of mito-targeted drugs: (A) TPP^+ -based conjugate drugs (B) peptide-based conjugate drugs (c)plastoquinone-based conjugate drugs.

Figure 7

Reliable synthetic strategy to obtain mitochondriotropic compounds, for the delivery of resveratrol (A) or SkBQ (B) into mitochondria.

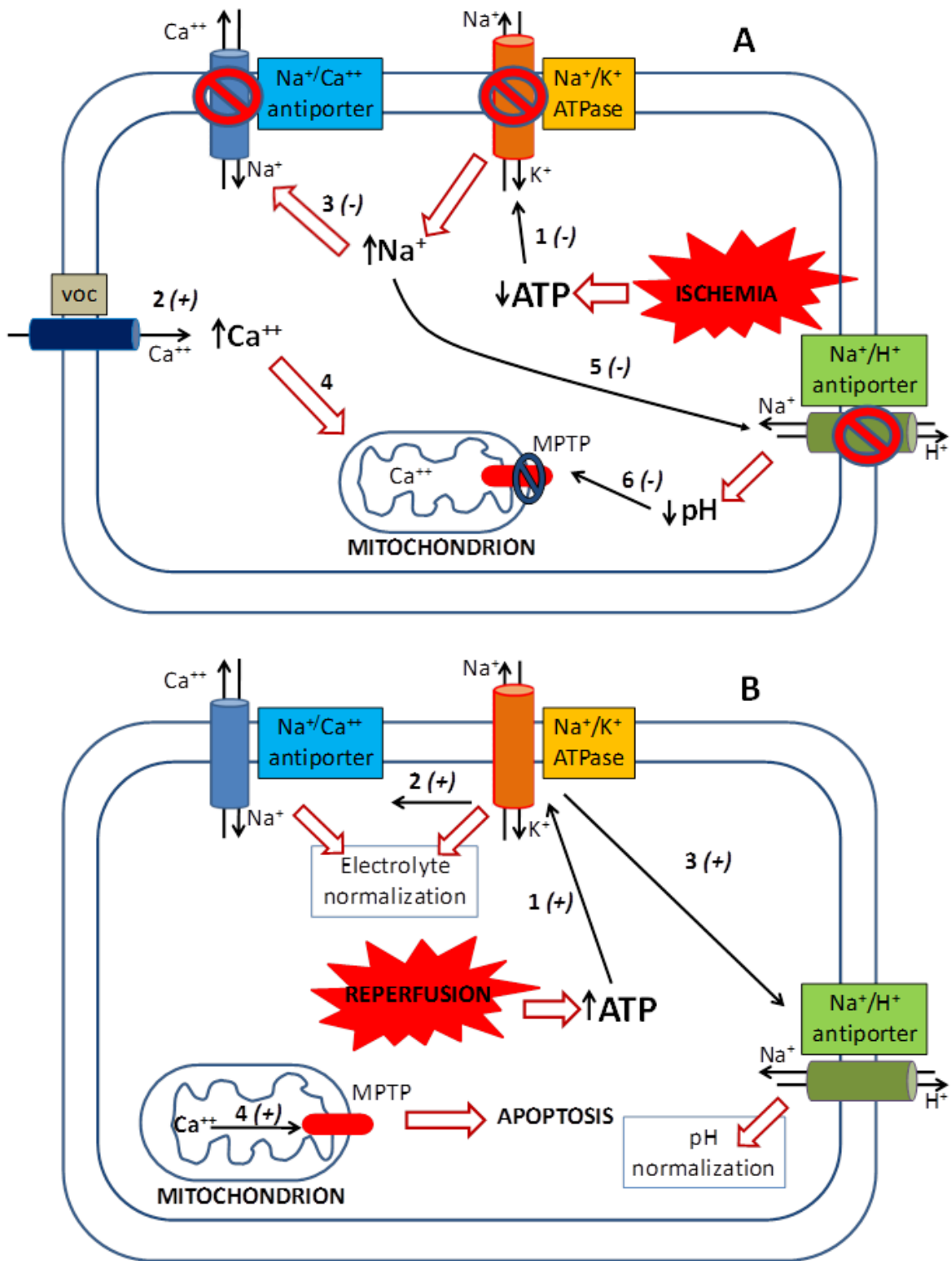


Fig. 1

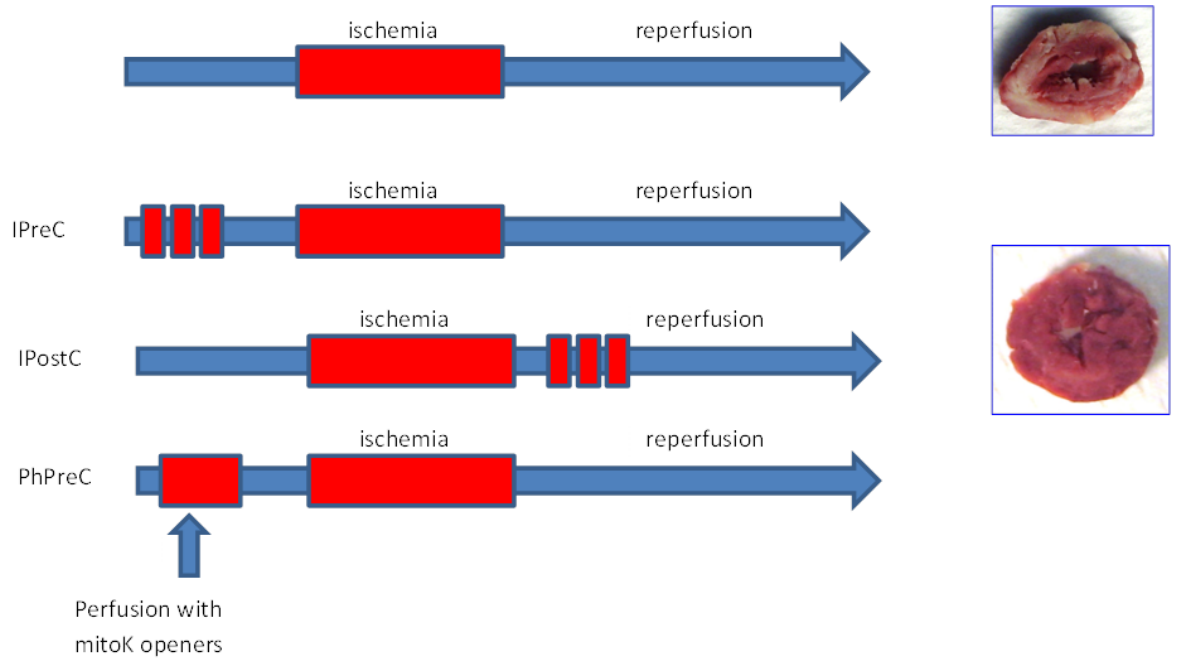


Fig. 2

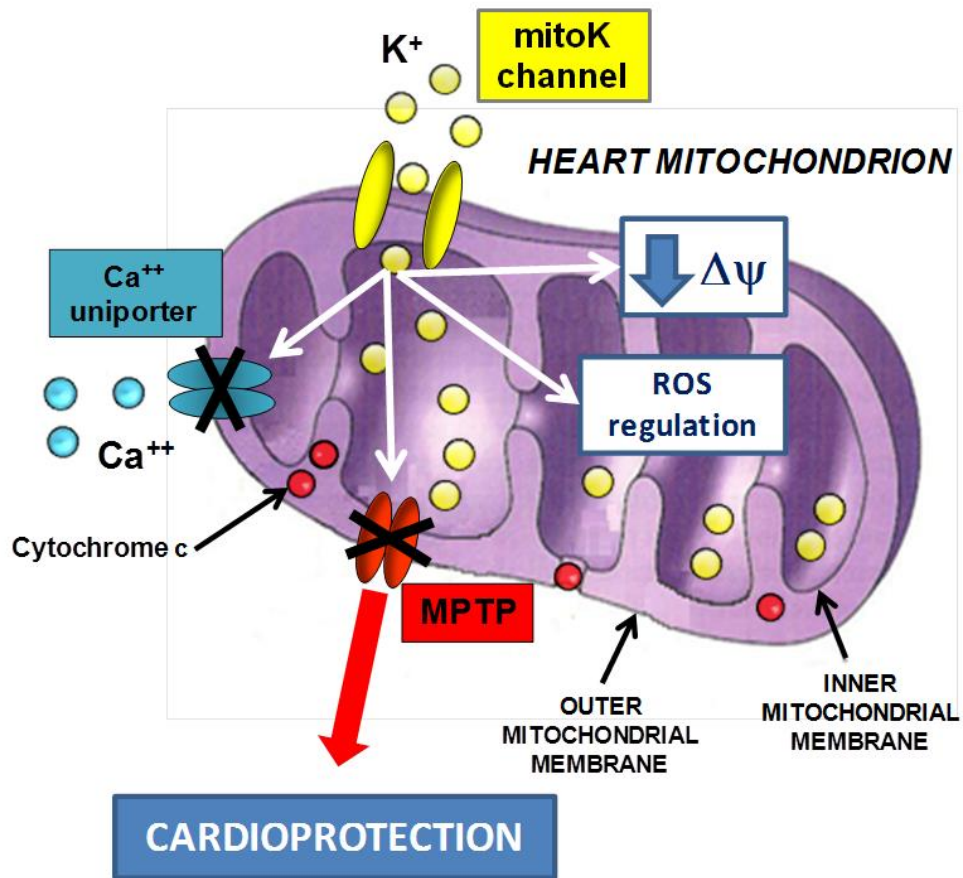


Fig. 3

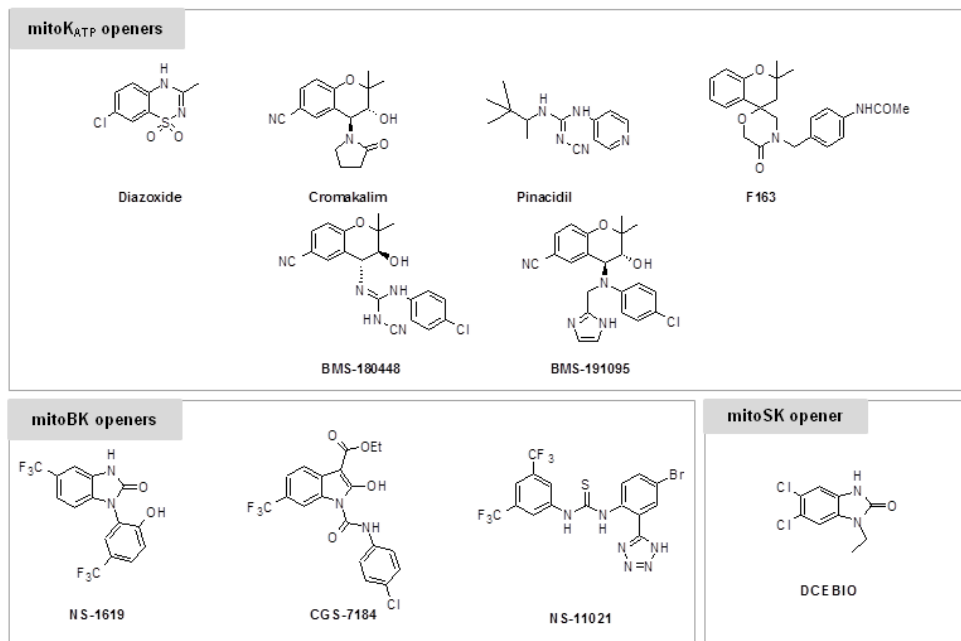


Fig. 4

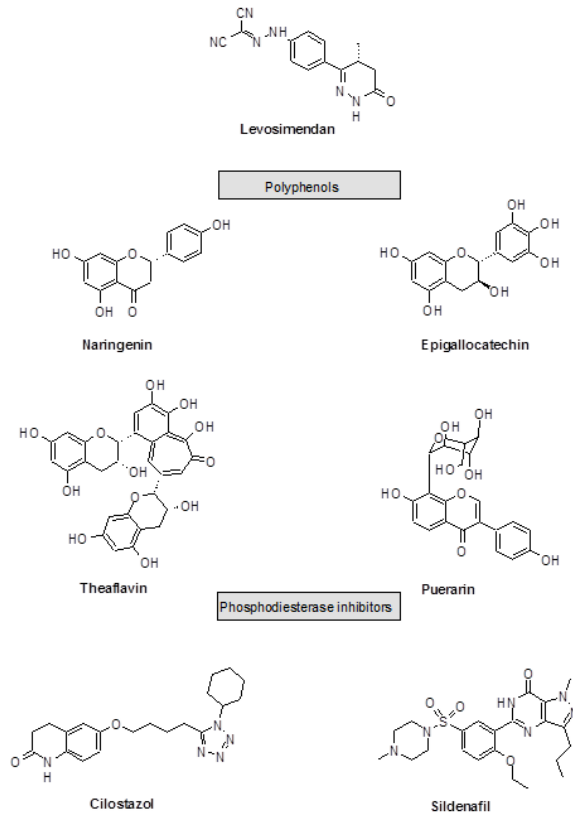


Fig. 5

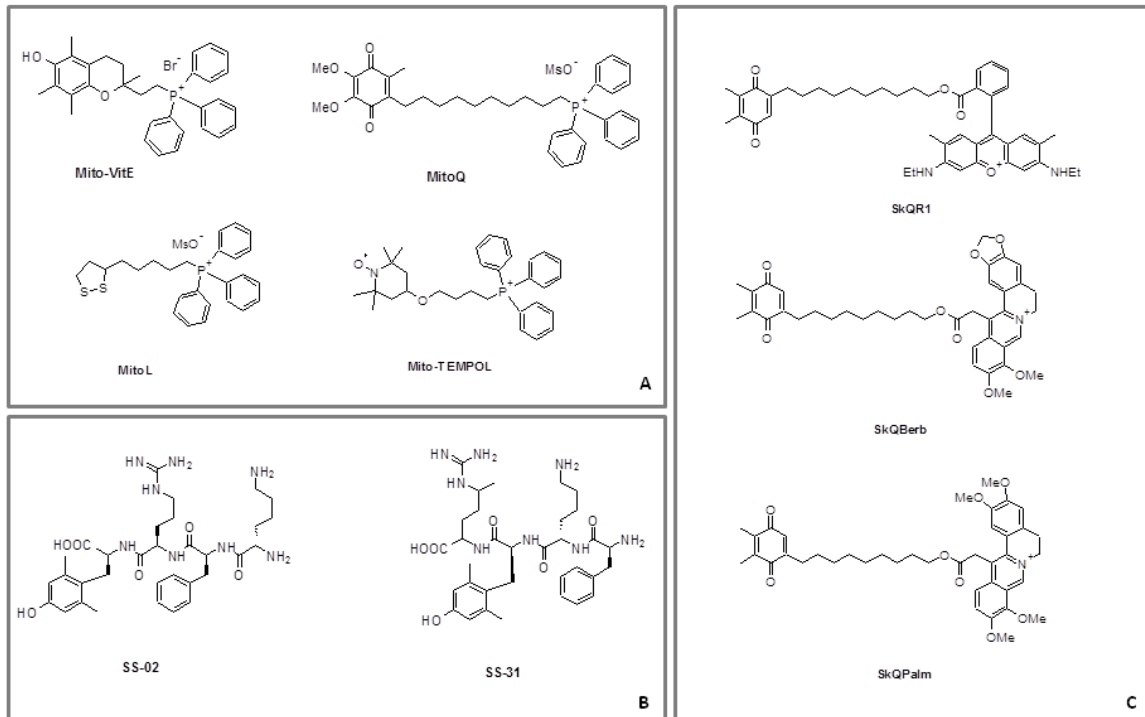


Fig. 6

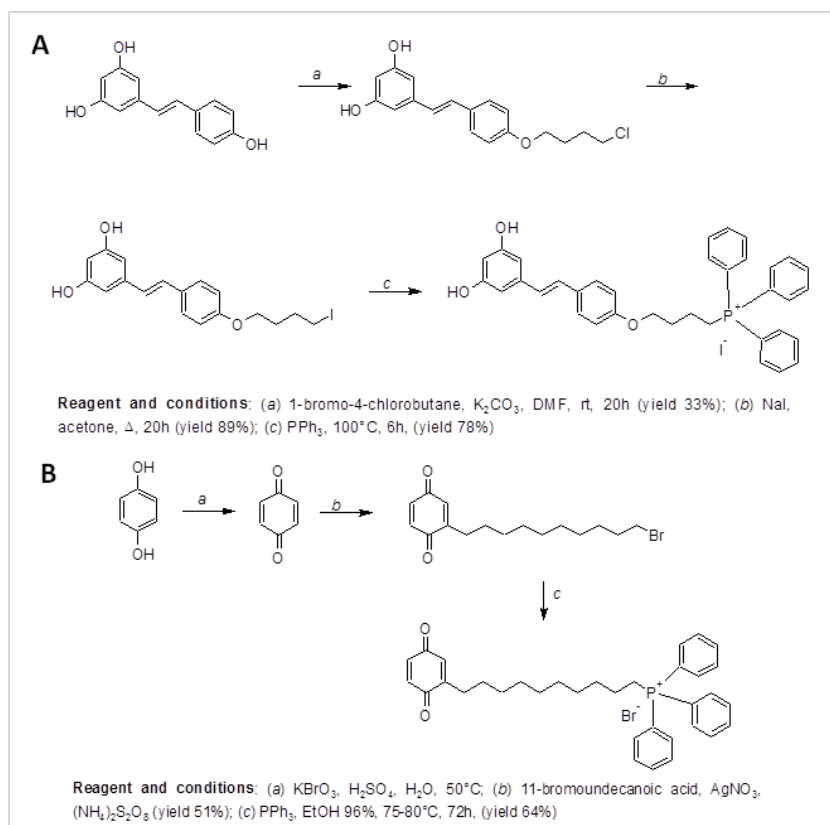


Fig. 7