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Mitochondrial Targeting for Drug Development

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1. Introduction

What is the reason that mitochondrion is so attractive target for pharmacotherapy? The problem raised is a bit complex. Although the appearance of mitochondria in animal cells started more than one million years ago, when the proteobacteria (in particular, Rickettsiales or close relatives) entered and practiced coexistence inside eukaryotic cells via endocymbiosis, But the biological science started to pay attention on mitochondria in the middle of 19th century, the time that mitochondria were discovered in tissue section of liver and flight muscle. The earliest definition of this organelle in cells was written by Richard Altmann on 1890, when he named them as “bioblasts” and hypothesized that they were “elementary organisms” living inside cells with “vital functions” [1]. After 1890, the progress in understanding the structure and function of mitochondria was quite steady, but we can single out major milestones in every decade. In the 1950s, investigators analyzed mitochondria by electron microscopy and characterized that they are the sites of respiration, Oxidative Phosphorylation (OXPHOS) and fatty acid oxidation. In the 1960s, investigators found out mitochondrial DNA (mtDNA) and described chemiosmotic theory. In the 1980s, the first consummate sequence of mammalian mtDNA and the first molecular identification of a cause of mitochondrial diseases were reported. A great incrementation in interest on mitochondria occurred in 1996, when researchers demonstrated that the organelles are associated with programming cell death or apoptosis [2]. After this manifestation, mitochondrial study commenced growing as a biomedical field. Owing to the unique structure and function of mitochondria, several clinically used drugs can improve or damage their bioenergetics. These drugs can act via the regulation of: (a) permeability transition pores (PTPs), (b) fatty acid uptake or oxidation, (c) the electron transport chain (ETC), (d) cardiolipin (CL) content (e) ion channels and transporters, (f) Adenosine triphosphate (ase) (ATPase) and (g) mtDNA and protein synthesis [3]. Mitochondria are sub-cellular organelles that play pivotal roles essential for energy (ATP) production, metabolism,

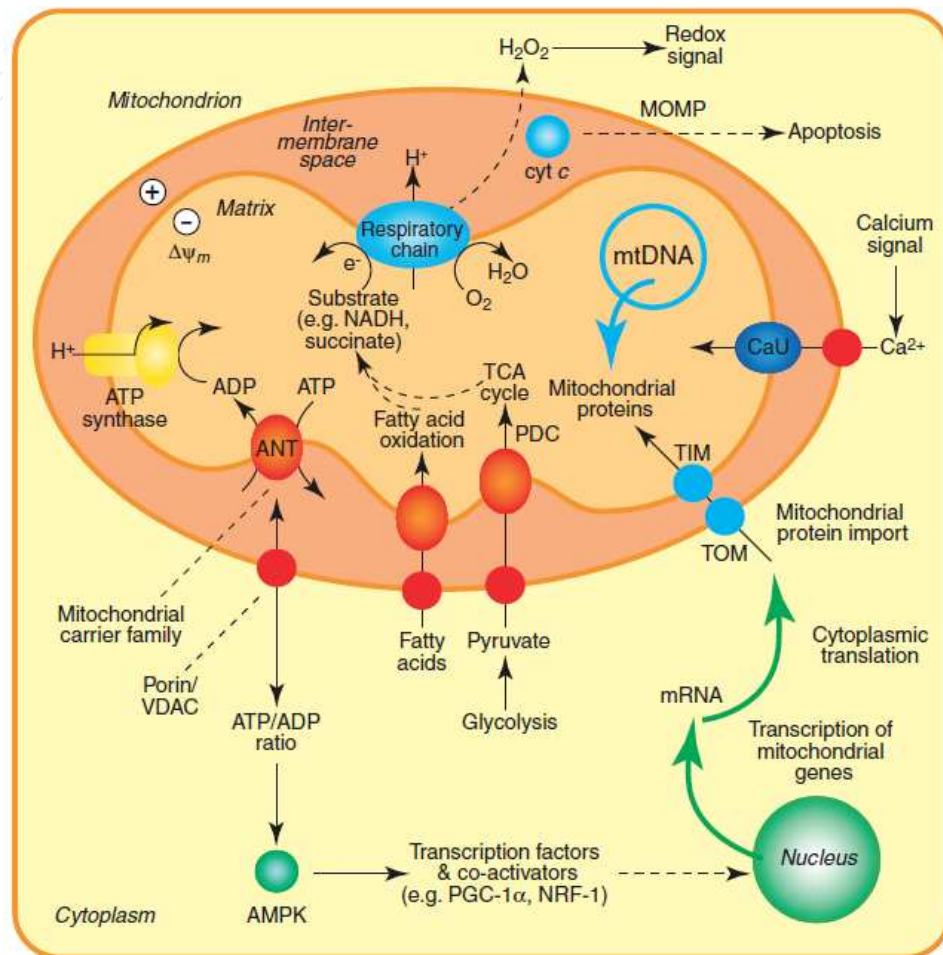
and homeostasis. In addition, mitochondria orchestrate some survival and cell death signaling. The reason why the mitochondria is considered as a potential drug target for the treatment of hyperproliferative and metabolic disorders. Unsimilarities in the reduction/oxidation condition of tumor versus non-tumor cells may be beneficial to get selective cytotoxic and anti-colonygenic effect on tumor cell populations. It was shown that pro-oxidant drugs, including Elesclomol and Trisenox have therapeutic benefits in the treatment of cancer. Findings obtained with Bz-423 in mouse demonstrate the potential for mitochondria-targeted drugs to control disorders of immune function. Investigation associating an elevated oxidant state with mitochondrial damage, aging dictates, and degenerative disease the need for a better understanding of how and when pharmacological manipulation of mitochondrial function prepares most therapeutic benefit [4].

Mitochondria carry out vital biochemical functions essential for cells such as homeostasis calcium, cell death and survival, in addition to ATP production. They represent a convergence point for death signals triggered by both intracellular and extracellular cues. Not surprisingly it's incoherent, therefore, mitochondria additionally offer targets for xenobiotics to exert either detrimental or therapeutic effects on cell survival and function. Efforts to harness mitochondrial targets for therapeutic benefit have focused largely on cancer, although treatments for ischemia, metabolic diseases and neurodegenerative diseases also are being explored. This chapter will describe current thinking and recent advances in the discovery of small molecule drugs acting on targets in the mitochondrion [5].

2. Why we choose mitochondria for drug targeting

The mitochondrion is as a respiratory organelle exists in almost all eukaryotic nucleated cells. Its unique structure is consisted of four distinct sub-structures with different specific functions: the mitochondrial matrix, the inner mitochondrial membrane (IMM), the outer mitochondrial membrane (OMM) and the intermembrane space (IMS). The structure of the inner mitochondrial membrane (IMM), is extensively folded and compartmentalized. The numerous invaginations of the membrane are called cristae, which house the 4 complexes of the mitochondrial respiratory chain and ATP synthase, controlling the vital levels of cellular bioenergetics. This primary function of the mitochondrion is responsible for supplying cellular energy, the reason why we call it power plant of the cell." However, it is not the only important function of mitochondria in the cell [6]. Adenosine triphosphate (ATP) production through the oxidative phosphorylation (OXPHOS) process requires a continuous flow of electrons. As such, mitochondria are the major so are the major source of reactive oxygen species (ROS, i.e. superoxide and H_2O_2), generated as byproducts of the ETC. ROS reflect the level of cellular oxidative stress, causing severe damage to macromolecules when overproduced. Consequently, according to the Harman's oxidative stress theory, they have been linked to aging, age-related pathologies, and death. However, when produced in a controlled amount, ROS may also play important signaling roles in various redox-dependent processes, including apoptosis, cell proliferation and hypoxia. Furthermore, mitochondria are active players in cellular calcium homeostasis. Mitochondrial Ca^{2+} accumulation regulates functions as diverse as aerobic metabolism and induction of cell death. Finally, mutations in mitochondrial DNA (mtDNA) are responsible

for many mitochondrial metabolic disorders, and are thought to contribute to aging by promoting apoptosis. Thus, because of their pivotal role in regulating cell life and death, mitochondria represent an attractive target for mitochondrial gene therapy as well as drugs treating either degenerative or hyper proliferative diseases (figure 1) [7].



Some aspects of mitochondrial biogenesis and some important roles of mitochondria in cell function are illustrated. One major function of mitochondria is the production of energy (ATP) via aerobic metabolism of glucose which is called oxidative phosphorylation. Initially, glucose is metabolized to pyruvate through cytosolic glycolysis (anaerobic metabolism). Voltage-dependent anion channels (VDACs), also called porin channels, allow low molecular weight molecules to enter the mitochondrial inter membrane space. Following the activation of AMPK (AMP-dependent kinase), cell responds to the decreased ATP/ADP ratio in the cytosol through alterations in AMP, and affects several targets. Mitochondrial mtDNA encodes 37 genes that are involved in the synthesis of the respiratory chain and the ATP production. Additional proteins are imported through TIM (transporter inner membrane) and TOM (transporter outer membrane), translocases of the inner and outer membranes that transport nuclear-encoded proteins into mitochondria. The adenine nucleotide translocase (ANT) enables the mitochondrion to import ADP and export ATP. Mitochondria also contribute to calcium homeostasis by taking up calcium into the mitochondrial matrix through the calcium uniporter in response to changes in cytosolic calcium. In addition, mitochondria play a crucial role in cell death such as apoptosis and necrosis. When apoptotic signals occur, the outer membrane becomes compromised and the mitochondrion experiences mitochondrial outer membrane permeabilization (MOMP), leading to the release of cytochrome c (cyt c) and many other pro-apoptotic proteins from the intermembrane space into the cytosol where they activate apoptotic cell death [6].

Figure 1. Mitochondrial biogenesis and function.

3. Mitochondrial diseases

Mitochondrial dysfunction triggers the cell death signaling cascade and results in organ failure and disease. Therapeutic intervention at the mitochondrial level can be envisioned for general cell-degenerative as well as hyper proliferative diseases, i.e. cancers. Hyper proliferative cells are sensitive to pro-oxidant that induced apoptosis through increasing of their oxidative stress level. The redox status of many tumors is significantly changed compared with normal tissue, and pro-oxidant drugs can use this difference for treatment of proliferative disorder. Conversely, degenerative and aging diseases are associated with an elevated oxidant state that may associate mitochondrial damage. In these cases, antioxidants targeting mitochondria are hoped to exert a justifying effect. Several studies are found in this category, all sharing the common features of disturbances of mitochondrial ROS, ATP or Ca^{2+} metabolism. They contain cardiovascular diseases (for example atherosclerosis, ischemia/reperfusion injury, heart failure, stroke); aging and neurodegenerative diseases (for example Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS) and Friedreich's ataxia (FRDA)); chronic autoimmune inflammatory diseases (for example rheumatoid arthritis (RA)) ; metabolic diseases(for example diabetes and obesity) ; as well as ionizing radiation injury (Table 1) [6].

Cardiovascular diseases	Neurodegenerative diseases	Chronic autoimmune inflammatory diseases	Metabolic diseases	Cancer
Atherosclerosis	Alzheimer's disease (AD)	rheumatoid arthritis (RA)	diabetes	Hepatocellular carcinoma
Ischemia/reperfusion injury	Parkinson's disease (PD)		obesity	Adenocarcinoma
Heart failure	Huntington's disease (HD)			Breast cancer
Stroke	amyotrophic lateral sclerosis (ALS)			Prostate cancer
Congestive heart failure	Friedreich's ataxia (FRDA)			

Table 1. The table above contains some disease that role of mitochondria in these diseases has been demonstrated. Which are divided into five categories.

4. Strategies to target mitochondria

Small molecule drugs or biologics can act on mitochondria through various pathways. Many of these mechanisms will be argued in more detail in the below sections, and a detailed discussion would immensely encroach the purpose of this chapter, but attractive current approaches include OXPHOS uncoupling, mitochondrial Ca^{2+} modulation, ETC inhibition and

control of oxidative stress through increase or decrease of mitochondrial ROS accumulation. The inhibition of the ETC can happen through direct inhibition of a protein subunit of one or more of the enzyme complexes or via reception of electrons current across the ETC instead of the natural receiver cytochrome c or ubiquinone. In the Oxidative Phosphorylation (OXPHOS) uncoupling occurrence, protons are shifted from the mitochondrial matrix to the intermembrane space (IMS) and do not avert across the F1F0-ATPase and back to the matrix, but instead migrate directly across the inner mitochondrial membrane (IMM). This bypass results in lack of ATP formation but heat production. Typical instances for agents that elevate OXPHOS uncoupling are weak bases and weak acids, which can be protonated in the IMS and carry protons across the IMM. Interestingly, compounds affecting the activity of inner membrane uncoupling proteins (UCPs) can inhibit cell death. An important occurrence starting the apoptotic cascade is the mitochondrial membrane permeabilization (MMP), which begins the collapse of the mitochondrial potential ($\Delta\Psi$), the release of cyt c and other protease and nuclease activators. The inhibition of this process can be attained with inhibitors of the mitochondrial permeability transition pore (mPTP) complex, openers of the mitochondrial ATP-regulated (mitoKATP) or inhibitors of the mitochondrial Na^+ - Ca^{2+} exchange or Ca^{2+} -activated (mitoKCa) potassium channels. Modulation of mitochondrial Ca^{2+} can also be envisioned by interference with mitochondria-specific Ca^{2+} transporters. Additional strategies for drug-induced perturbation of mitochondrial biochemistry include the inhibition of the cyt c-catalyzed peroxidation of the mitochondria-specific phospholipid CL, and the targeting of other specific mitochondrial proteins via inhibition of kinases, F1F0-ATPase, enzymes of the Krebs cycle, or members of the anti-apoptotic Bcl-2 family. It has been known for a while that inhibition of the oxidative cellular damage through a decrease of mitochondrial ROS accumulation can be attained by the delivery of antioxidants acting as radical and/or electron scavengers. Many compounds are able to inhibit the β -oxidation of unsaturated fatty acids, causing cellular accumulation of fat. Alternatively, anti-apoptotic agents could be designed via inhibition of the cyt c-catalyzed peroxidation of CL. Finally, the mitochondrial biochemistry is also severely derailed by mtDNA binding/oxidation or inhibition of mtDNA synthesis, or modulation of mitochondrial fission/fusion. Chemical agents that bind to mtDNA often result in inhibition of DNA synthesis. If adequate selectivity in the binding process can be achieved, this mechanism of action may display an attractive strategy to block the expression of mutated mtDNA accountable for genetic mitochondrial disarrays. Lately, compounds that modulate mitochondrial fission/fusion have been suggested as a valuable replacement in treatment of neurodegenerative diseases (figure 2) [6]. While the OMM is relatively permeable due to the abundance of the VDAC protein, the IMM is extremely impermeable and acts as a stiff barrier to the passive propagation of all types of molecules. It is also wealthy in the unusual phospholipid cardiolipin (CL), and keeps a strong negative internal potential of -180 mV needed for the ETC function. A widely used strategy for targeting mitochondria takes benefit of this considerable biophysical membrane nature, since cationic molecules are attracted to and accumulate preferentially within the negatively charged mitochondrial matrix. Another strategy is based on the access of an agent to mitochondrial membrane components, particularly to the phospholipid cardiolipin CL which is particularly found in the IMM. Moreover the former more specific properties, adequate lipophilicity is also needed to achieve an adequate enrichment in mitochondrial compartments. A rising approach to the selective delivery of bioactive cargo molecule into mitochondria uses a carrier of short peptide sequences with

specific physicochemical properties. For example, Horton *et al.* newly reported such mitochondria-penetrating peptides with changing cationic and hydrophobic residues. Other variants have been based on an oligomeric carbohydrate scaffold, always attaching key guanidinium moieties due to their delocalized cationic form. Finally, the tethering of active molecules to mitochondrial targeting sequences (MTSs) has also been successively utilized. Mitochondrial targeting sequences (MTSs) are peptides applied by cells for the delivery of nuclear-encoded mitochondrial proteins, comprising structural motifs recognized by the mitochondrial import machinery. Another class of mitochondrial delivery vectors, appropriate for the import of impermeable or big molecules, is the vesicle-based transporter system. The targeted agent is encapsulated in a cationic liposome, which undergoes cellular internalization and subsequent fusion with the OMM. In summary, by the utilization of a wide range of various delivery systems, the targeting of mitochondria for therapeutic advantages can be employed to enrich both pro-oxidants as well as antioxidants in mitochondrial compartments. Antioxidants are of preliminary interest for their antiaging properties, with some of the main applications centered neurodegenerative and cardioprotection diseases, while cytotoxic and pro-oxidant agents are under research for cancer therapy [6].

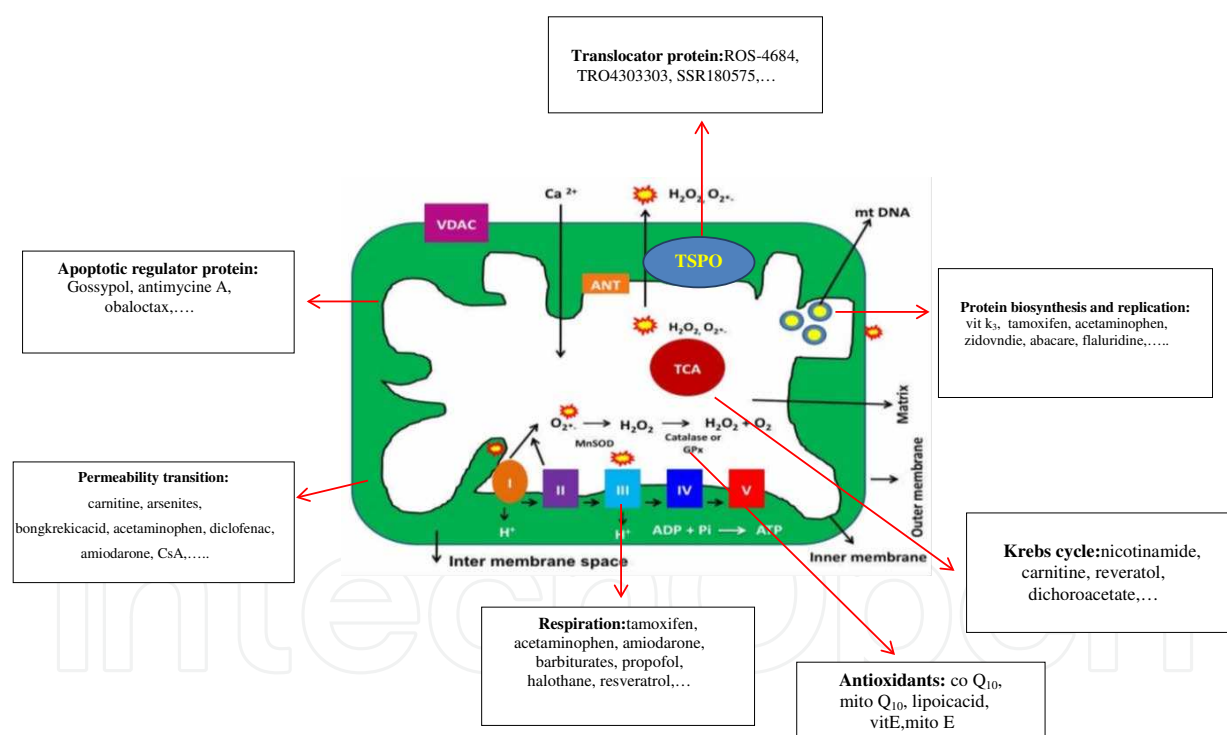


Figure 2. Pharmacological targeting of mitochondria of possible sites of drug action.

4.1. Targeting the mitochondrial electron transport chain

Mitochondria are unusual organelles. They act as the power plants of the cell, are surrounded by two membranes, and have their own genome. The mitochondrion consists a matrix encircled by two membranes, the MOM and the MIM. The MIM comprises several invagina-

tions called cristae and is very impermeable to ions and small molecules, which need specific transport proteins to exit or enter the mitochondrial matrix. Under aerobic statuses, the proteins of the ETC, placed in the MIM, reduce oxygen to water through a series of steps along the electron transport chain that use NADH and FADH₂ derived from the glycolysis and tricarboxylic acid cycle. These reductions effectively efflux protons (H⁺) through the MIM such that they accumulate in the IMS creating a pH gradient across the MIM that contribute to an overall electrochemical gradient (DC). This gradient as a source of energy to drive the synthesis of ATP from ADP and phosphate is applied by the mitochondrial F₁F₀-ATPase. This succession of chemical stages is collectively known as OXPHOS.

Small amounts of ROS are generated as a result of incomplete oxygen reduction in during normal OXPHOS. ROS comprise superoxide, the result of partial oxygen reduction, hydroxyl radicals and the subsequently formed hydrogen peroxide, each of which displays different chemistry. A high NADH: NAD⁺ ratio (as may arise owing to high rates of glycolysis) can increase ROS production, as does state 4 respiration in which electron transport occurs in the loss of ATP synthesis, for instance, when ADP levels are low [8]. Inhibitors of the ETC and of the F₁F₀-ATPase can also enhance mitochondrial ROS production. ROS act as secondary messengers with important signaling roles, but in addition ROS contribute to oxidative damage of cellular macromolecules. It is also remarkable that the production of ROS has been recognized as a widespread mechanism for the bactericidal effect of many widely used antibiotics including drugs targeting DNA, the cell wall and protein synthesis [9]. Thus, ROS perform both a destructive role and necessary role in cells. Inhibitors of the electron transport chain are useful tools for furthering our understanding of this essential bioenergetics process [10]. Inhibitors of complex I (NADH ubiquinone oxidoreductase) include the photochemical Annonaceousacet ogenins that have been attributed with antimicrobial and anticancer properties and rotenone used as a rodenticide. The widely used diabetes drug metformin inhibits complex I and has been shown to induce AMP-activated protein kinase-dependent and p53 increase in glycolysis to countervail for modulation of the respiratory chain, which effectively increases glucose utilization. Succinate-ubiquinone oxidoreductase (Complex II) is one proposed target of redox-silent vitamin E analogs such as α -tocopheryl succinate. Cytochrome c oxidoreductase (complex III) is inhibited by the natural product myxothiazole and by antimycin A (the active constituent of the piscicide Fintrol). Cytochrome c oxidase (complex IV) is a target of cyanide. Complex I and complex III are the main sources of mitochondria derived ROS in vitro, although the synthesis of superoxide by complex III is considered to be more physiologically related. The electron transport chain provides the H⁺ gradient that is necessary for the mitochondrial F₁F₀-ATPase to function. The related macrolide apoptolidin and Oligomycin, a natural product that blocks the proton channel are both inhibitors of the F₁F₀-ATPase. Apoptolidins display remarkably selective cytotoxicity toward a subset of tumor cell lines in vitro, suggesting that inhibition of the ATPase is not exactly cytotoxic. Other compounds reported to bind to the F₁F₀-ATPase include aurovertin, resveratrol, PK1119, Bz-423, and diindolyl methane (DIM) [11]. The benzodiazepine derivative Bz-423 was identified as a lead for the treatment of autoimmune diseases. Bz-423 reduces disease in murine models of lupus, psoriasis, and arthritis and has cytotoxic and anti-proliferative effects on tumor cells in vitro. Bz-423 is an uncompetitive inhibitor of the F₁F₀-ATPase,

deceleration the ATPase without causing a significant drop in cellular ATP levels. The therapeutic effects of this compound are moderated by the induction of superoxide O_2^- . Resveratrol, a constituent of grape skins, increases longevity in rodents and has been attributed with beneficial effects against inflammation, heart disease, and cancer. Notwithstanding the existence of a crystal structure of resveratrol bound to the F1F0-ATPase, this protein is one of several reported targets for resveratrol and related compounds, including the protein deacetylase, sirtuin [5].

The ETC and the F1F0-ATPase proteins can be decoupled by uncoupling proteins that promote the leakage of protons back through the MIM. The resulting drop in membrane potential reduces ROS production and represents a natural protective mechanism against inhibition of respiration. This is a natural process that results in thermogenesis. F1F0-ATPase inhibitors, without affecting ATP synthesis, specifically block ATP hydrolysis have been described: such compounds should be effective under ischemic conditions when the ATPase can operate in the reverse of its normal direction leading to a catastrophic drop in ATP levels that causes cell death [12]. This premise has not been tested clinically. Mammalian and bacterial ATP synthases exhibit substantial differences in structure and intracellular location presenting the opportunity for species selective ATP synthase modulation [5]. The mycobacterial ATP synthase inhibitor, R207910, is currently in Phase III trials for the treatment of tuberculosis [13].

4.2. Targeting transporters and channels in mitochondria

It is well recognized that the totality of the mitochondrial membrane is crucial for mitochondrial function. Not only are the inner and outer membranes targeted by drugs, but, in addition, many of the ion channels, proteins, and transporters embedded within the lipid membrane are also targeted. Among the main drug targets are: 1) lipophilic cations targeting the IMM (e.g., rhodamine-123) 2)cardiolipin(CL) (e.g., 10-N-alkyl-arcine orange), 3) carnitine palmitoyltransferase- 1 (CPT-1) inhibitors (e.g., oxfenicine, perhexiline, and etomoxir), 4) Na^+ / Ca^{+2} exchanger regulators, 5) B-cell lymphoma 2 (Bcl-2) protein inhibitors (e.g., gossypol) 6) IMM potassium channel regulators (e.g., glibenamide and diazoxide), and 7) MPT pore complex regulators (e.g., CsA). We can activate permeabilization of the mitochondrial membrane or can protect membrane integrity. Among the best mitochondrial protein targets for many drugs are a group of proteins that form the PTP complex across the OMM and IMM. This complex is responsible for mitochondrial permeability transition and plays a crucial role in both survival and death signaling pathways. Depending on the pharmacological strategy, MPT pore activation stimulates apoptosis and prevents the differentiation of many tumor cells. Strategies to induce this effect typically involve direct action against the MPT pore protein complex or indirect action via depleting endogenous inhibitors of MPT pore or increasing ROS and calcium ions in the cytoplasm. Various MPT pore complex inhibitors, in anticancer therapeutic approaches, are used, including: 1) hexokinase modulators such as glucose-6-phosphate and glucose 2)creatine kinase modulators such as cyclocreatine and creatine; 3) cyclophilin D (CypD) -affecting drugs such as sangliperin A and CsA; 4) voltage dependent ion channel modulators such as arsenic trioxide; 5) benzodiazepine receptor modulators such as Ro-54846 and PK11195; and 6) adenine nucleotide translocase modulators such as CD437, PENAO (4-(N

(Spenicillaminylacetyl) amino) phenylarsonous acid), lonidamide, betulinic acid, clotrane, and bongkrelic acid, GSAO (4-[N-[S-glutathionylacetyl amino] phenylarsenoxide)[14], GSAO and PENAO are tumor-metabolism inhibitors that target ANT of the inner-mitochondrial membrane. Both the compounds are currently being appraised in trials in patients with solid tumors. The trivalent arsenical moiety reacts with the two matrix-facing cysteine residues of ANT, inactivating the transporter. This leads to tumor-supporting cells and death and proliferation arrest of tumor cells [14]. Above-mentioned drugs grouping although useful appears to be synthetic, and surely will be modified. According to some authors MPT pore may consist of quite different proteins. Recent investigation on MPT pore molecular identity has to redefine a new context on described interaction. CL, a negatively charged phospholipid, is almost exclusively localized in the mitochondrial inner membrane. CL maintains architecture and membrane potential. A loss of CL content has been associated with mitochondrial damage in multiple tissues in a variety of pathological conditions, including aging, heart failure, and ischemia. It was reported that preadministration of NAO (10-N-alkyl-arcine orange), that is a dye associated specifically with CL, decreased the release of cytochrome c, a component of the ETC in mitochondria, released in response to pro-apoptotic stimuli [15]. Another drug target example is CPT-1, an enzyme located in the OMM and responsible for the transport of long-chain fatty acids across the membrane by binding them to carnitine. Perhexiline and etomoxir (antianginal agents) act by inhibiting CPT-1 and protect heart from fatty acid-induced ischemic injury [16].

In contrast to the MIM, the mitochondrial outer membrane is more permeable to small molecules so that the IMS resembles cytosol in its small molecule composition. In addition, however, the IMS sequesters proteins such as apoptosis inducing factor (AIF), smac/ Diablo (second mitochondria derived activator of caspases), and cyt c that when released into the cytosol activate caspases and induce apoptosis. One process for the release of these death inducing protein factors involves swelling of the mitochondrion so that the outer membrane ruptures producing MPT. These events are mediated by the MPT, a channel that comprises multiple proteins including the VDAC located in the MOM, ANT located in the MIM, as well as the peripheral benzodiazepine receptor (PBR), CypD, hexokinase, and possibly also Bax and Bcl-2. Inhibitors of the MPTP have been reviewed elsewhere as have inhibitors of Bcl-family proteins. High affinity ligands of the PBR have been associated with immunotherapeutic and anticancer properties. The relationship of these effects to physiological functions of the PBR requires more study. Newly, VDAC ligands identified in cell-based screens were shown to be cytotoxic toward cells bearing oncogenic Ras protein [4].

4.2.1. Targeting mitochondrial Adenine nucleotide translocator (ANT)

Adenine nucleotide transporter interacts with Voltage dependent anion channel (VDAC) and cyclophilin D and other proteins to make the mitochondrial permeability transition pore (mPTP) at locations where IM contacts OM [17]. Adenine nucleotide transporter (ANT), the key IM protein of mPTP, exchanges ATP and ADP. ANT can form mitochondrial permeability transition pores (mPTP) which induces other membrane leakiness of mitochondria and subsequent swelling of matrix. This event happens following the surface area of the IM (with its folded cristae) exceeds that of the OM. Quite in contrast, the conformation of ANT is

modulated by ANT ligands [18] and sensitive interaction of cyclosporine A with cyclophilin D, indirectly blocks VDAC activity. When reconstructed into planar lipid bilayers or into liposomes or into planar lipid bilayers, ANT can form nonspecific channels in response to proapoptotic agents such as the HIV-1 viral protein R (Vpr), Ca^{2+} , lonidamine and atractyloside. Moreover, ANT channel formation is inhibited by Bcl-2 and enhanced by Bax. However, mouse knockout studies led to the conclusion that ANT would not (always) be required for apoptotic MPT. Recent evidence suggests that some ANT isoforms (ANT1, ANT3) are apoptogenic while others are not (ANT2)[19]. In 2005, a fourth ANT isoform (ANT4) has been identified in mouse and man by means of two independent experimental approaches. ANT2, which are over expressed in cancer cells, help to stabilize mitochondrial membranes and survival cell. Indeed, it was suggested that in cancer cells, small interfering RNA (siRNA) that down regulate ANT2 may constitute a valid strategy for the selective induction of tumor cell apoptosis [20].

4.2.2. Targeting mitochondrial cyclophilin D

CypD is a nuclear-encoded mitochondrial isoform of cyclophilin, with a molecular mass of 18 kDa. It enters mitochondria using a targeting sequence that is cleaved following translocation into the matrix. At present, extensive data have been obtained in favor of Cyclophilin D as an essential component and key regulator of MPT pore using various pharmacological inhibitors and genetic manipulations. The first evidence for the involvement of Cyclophilin D in MPT pore formation came from studies showing an inhibitory effect of CsA, extensively used in tissue and organ transplantation, as an immunosuppressant, on pore opening. Other document for the essential role of Cyp D in MPT pore formation has been reported by several independent groups in reports with Cyp D knockout mice in which mitochondria isolated from these animals displayed a low sensitivity to Ca^{2+} and, as a result, a delayed MPT pore opening. The inhibitory effect of CsA and its analogs involves interaction with Cyp D that reduces sensitivity of pore opening to Ca^{2+} . Cyp D favors MPT pore opening by facilitating the Ca^{2+} -triggered conformational change. Most probable, interaction of CypD and Ca^{2+} , P and the pore is a multifaceted process that also includes enhancement of susceptibility of the MPT pore proteins to oxidative stress [21].

Several studies have shown that Cyp D is up-regulated in many human tumors and can function as an apoptosis repressor. Growing number of evidence demonstrated that the anti-apoptotic regulation of Cyp D might be associated with the stabilization of hexokinase II binding to mitochondria. Inactivation of CypD with cyclosporine A or knock-down of the expression using siRNA was shown to release hexokinase II from mitochondria. Because Cyp D is a mitochondrial matrix protein, an intermediate in the IMM between the OMM and matrix is necessary for its modulation of hexokinase II binding to VDAC. ANT in the IMM could play this intermediation role. However, study showed the opposing pro-apoptotic role of Cyclophilin D in apoptosis. They demonstrated that hexokinase II detachment-triggering apoptosis might be associated with a disruption of the interaction of Cyp D with ANT. Furthermore, inhibition of CypD was shown to prevent the onset of the MPT pore [22].

The MPT pore, a critical mediator of cell death, has appeared as a serious therapeutic target for limiting acute ischemia reperfusion injury. The genetic amputation and pharmacological

inhibition of mitochondrial Cyp D, a key mediator of apoptosis signaling, has emerged as an important therapeutic target for minimizing acute hypoxic/ischemic injury. The genetic ablation and biological inhibition of mitochondrial cyclophilin-D (CypD), a regulatory component of the mitochondrial permeability transition pore (mPTP), has been reported to decrease myocardial infarction progression in *in vivo* studies. However, it is note worthy that CypD-deficient hearts are still susceptible to mPTP opening and cell death signaling occurred through mechanisms which are not dependent on CypD. Very recently, cyclosporin-A (CsA), an immunosuppressive therapeutic agent and biological inhibitor of CypD has been shown to reduce myocardial infarction progression and improve left ventricular function in ST-elevated MI patients undergoing primary percutaneous coronary surgery, given at reperfusion [23].

Animals lacking CypD display increased resistance to ischemic insults, muscular dystrophies, multiple sclerosis (MS), ALS, and AD, and the CypD inhibitor CsA and its analogs have displayed neuroprotective effects in several animal models of acute neurological damage and chronic neurodegenerative disease. Preserving the integrity of mitochondrial membranes through inhibition of mPT has been put forward as the central mechanism for the neuroprotective and cardioprotective effects of CsA, even though the drug has several pharmacological targets. It has also been suggested that CypD is downregulated in neurons during development, which would decrease the sensitivity of the MPT pore to calcium, and prohibit the use of CypD as a pharmacological target in disorders of the adult central nervous system (CNS) [24].

4.2.3. Targeting mitochondrial peripheral benzodiazepine receptor (PBR)

The elaborate structure of mitochondria is important for the normal performance of the organelle and as a potential therapeutic target. Two specialized membranes embed each mitochondrion, dividing the organelle into an inner IMS restricted by the OMM and the inner IMM. The OMM comprises many channels formed by the protein porin that makes the membrane relatively permeable. One of the membrane proteins is the peripheral benzodiazepine receptor (PBR). PBR is a small evolutionarily conserved protein involved in steroid synthesis and cholesterol transport; it is also a regulator of apoptosis. The PBR is also involved in OMM permeabilization by interaction with the pro-apoptotic Bcl family of proteins. However, OMM permeability maybe independent of MPT pore opening because blocking PBR with 4'-chlorodiazepam (CDZ) prevents against ischemia-induced cytochrome c release independent of damage to the IMM;4'-chlorodiazepam (CDZ)also reduces ischemia-induced arrhythmias. PBR is found in close association with the VDAC and additional components of the mitochondrial contact site. This close association also suggests that PBR-VDAC may serve as a target for modulating apoptosis and may have implications for drug design to treat such disorders as cancer and neurodegenerative diseases [20].

4.2.4. Voltage-dependent anion channel (VDAC)

VDACs, also known as mitochondrial porins that show 68% similarity between mice and humans. Among three VDAC isoforms, VDAC1 is the most widely expressed in mammals followed by VDAC2 and then VDAC3. Studies have found that VDACs are highly conserved.

Three isoforms of VDAC: VDAC1, VDAC2 and VDAC3 are reported. The additional exon in VDAC2 is believed to encode part of the 5'-UTR region. VDAC1 and VDAC2 are expressed in the skeletal muscles, heart, liver, and brain. There is also very low level expression of VDAC1 but only in the testes. VDAC3 is expressed in the spleen, lung, adrenal, ovary, liver, testicular tissue and kidney muscles. Voltage dependent anion channel (VDAC) function functions in the cell, including regulating mitochondrial shape and structural changes, regulating ATP transport, regulating calcium transport, regulating apoptosis signaling, regulating hexokinase interactions with mitochondria, regulating cell survival, growth, and fertility and maintaining synaptic plasticity through mitochondrial permeability in the transition pore. These functions have been found to be altered in cells from patients with mitochondrial and neurodegenerative diseases, leading to mitochondrial dysfunction. As well as, increasing evidence suggests that VDAC interacts with several cytoplasmic proteins, changes channel activity and VDAC closure and reduces VDAC channel conductance. It is believed that VDAC is constantly open in metabolic state. However, recent evidence suggests that VDAC closes intelligibly during apoptosis in unhealthy neurons. As a result, with its pores closed, mitochondria may not be able to uptake ADP, inorganic phosphate and respiratory substrates from the cytoplasm and to release ATP into the cytoplasm. The pro-apoptotic protein tBid has been found to promote the pore closure whereas anti-apoptotic protein Bcl2-XL has been found to prevent VDAC closure. VDAC displays to be involved in both anti - and pro - apoptosis aspects of mitochondria. VDAC channel conductance may be impaired in a couple different ways. (1) Phosphorylated VDAC may also interact with cytoplasmic proteins, leading to the blockade of mitochondrial pores. Recently, in a study of brain tissue from postmortem brains of patients with AD, Reddy and Manczak found that VDAC interacted with mutant AD proteins, which in turn blocked mitochondrial pores and interrupted the flow of ADP, ATP, respiratory substrates and inorganic phosphate substrates between mitochondria and the cytoplasm, ultimately leading to mitochondrial dysfunction. (2) In neurons from mitochondrial diseases, VDAC may interact with cytoskeletal and mutant proteins that may have accumulated during disease progression and may have blocked the mitochondrial pores [25].

A lot of literature testes the role of VDAC in the regulation of cell death. VDAC is being studied as a cancer-specific target because tumor cells have increased VDAC expression and glycolysis. The role of VDAC1, VDAC2 and VDAC3, in cell death is intricate, but importantly, in vivo evidence shows that in cancer cells, the association of VDAC1 with HK prevents against mitochondrial-mediated apoptosis. Therefore, disruption of the VDAC1-hexokinase (HK) complex exhibits an attractive therapeutic cancer target. Over expression of HK1, 2 and their connection with VDAC are notable characteristics of glycolytic cancer cells. It was found that the VDACs expression has been elevated in cancerous cells compared with normal cells and could be altered with chemotherapy. Increased VDAC concentration is an unfavorable prognostic factor; moreover, RNA interference induced VDAC down regulation inhibits cancer growth. This evidence seems in contrast with the finding that over expression of VDAC induces apoptosis, but it illustrates how the context may influence the functional meaning of a biological parameter. In cancer up regulation of VDAC goes hand in with HK2 up-regulation and can be considered a component of glycolytic up-regulation. HK2 binding to VDAC, which allows for ATP transport out of mitochondria, leads to a cancer cell metabolic advantage

(termed the Warburg effect), and it antagonizes cell death through the inhibition of Bax-induced cytochrome c release and/or inhibition of the MPT pore [26].

4.2.5. Changes in the configuration of MPT pore as a target in treatment

Cellular redox potential can be changed by function of OXPHOS proteins as well as by the proliferative state. Elevations in intracellular oxidant potential can have discrete chemical consequences: for example, a pair of cysteine thiols in the ANT becomes oxidized to a disulfide linkage that results in opening of the MPT pore. Thus, manipulating cellular redox represents an approach to altering mitochondrial function. Arsenic trioxide is currently marketed for the treatment of acute promyelocytic leukemia. Its mechanism of action is undoubtedly multifactorial but is understood to involve the formation of disulfide linkages in mitochondrial proteins, including members of the MPT pore leading to their inhibition and the production of ROS [27]. Elesclomol (STA-4783), an injectable drug currently undergoing Phase III clinical evaluation for the treatment of metastatic melanoma, selectively kills cancer cells through apoptosis as a result of an increase in their already raised oxidant level [28].

4.3. Superoxide dismutase (SOD) as a target in mitochondria

Superoxide dismutase (SOD) represents a group of enzymes that use as cofactor zinc and copper, or nickel, iron, or manganese ions. There are three major families of superoxide dismutase, depending on the metal cofactor: The Ni type, which binds nickel (only in prokaryotes) and Cu/Zn (which binds both copper and zinc), Fe and Mn types (which bind either iron or manganese). SOD1 is located in the cytoplasm, SOD2 in the mitochondria, and SOD3 is extracellular. The first is a dimer, whereas the others are tetramers (four subunits). SOD2, the mitochondrial enzyme, has manganese in its reactive site whereas SOD1 and SOD3 contain copper and zinc. [28]

A key role in oxidative stress protection is played by the manganese containing SOD2 in mitochondria. This enzyme is also critical for fetus growth and viability in many eukaryotic organisms, since complete loss of the enzyme results in neonatal lethality in mice. In addition to oxidative stress nitrosative stress can completely inactivate mitochondrial Mn-SOD as well, possibly through nitration of a single tyrosine residue (Tyr-34). Consequently, this favors peroxynitrite generation in mitochondrion. Tyrosine nitration induced Mn-SOD inactivation being identified in more than 50 human diseases including ischemia/reperfusion, inflammation, human kidney allograft rejection and human pancreatic ductal adenocarcinoma [29].

The renal ischemia-reperfusion injury is one of the most important clinical examples in which Mn-SOD represents the main antioxidant protective mechanism. A significant increase in superoxide production is usually associated with Ischemia/reperfusion conditions which leads to a rapid depletion of SOD. Therefore, any external therapeutic involvement needs the sufficient amount of SOD to overcome the superoxide radical byproduct of ischemia-reperfusion conditions. Any therapeutic administration of exogenous SOD fails due to short half-life of the enzyme in plasma. A way to ensure a continuous production of SOD is entering SOD gene in renal tissue which guarantees protection from renal ischemia-

reperfusion injury. The effective gene delivery without toxic side effects was established by intravenous injection of the gene vectors during experiments on animal models before the ischemic insult. A significant progress in the area of kidney biology, especially in hereditary kidney disease and inflammatory and fibrotic diseases was achieved by the use of adenovirus as a vector for kidney-directed gene therapy [30]. Although some advantages make adenoviral vectors suitable for gene transfer into complex organs such as the kidney. But in contrast some disadvantages downgrade these vectors. For instance, the expression of the transfected gene is limited to weeks or months in this technique, because adenovirus does not integrate into the host genome. Secondly, the adenovirus can elicit immunological responses, therefore vector cannot be administered repeatedly. During emergency situations in other inflammatory renal disease states, the SOD gene therapy with adenoviral vector is recommended, however, occurrence of harmful effects maximum within a week is expected (e.g., post-transplant acute renal failure) [29].

ALS a neurodegenerative disease leads to paralysis, muscle wasting, and death, usually within 2 - 3 years of symptom onset due to death of motor neurons. The central mechanism by which motor neuron death occurs in familial ALS is oxidative stress which is due to the mutations in the antioxidant enzyme SOD1 gene. Many hypotheses studied so far using ALS mouse models. Some of these studies showed that SOD1 mutants have very low benefits (3, 35). One of the most important pharmacological outcomes obtained in ALS mouse models was increasing expression of either growth factors such glial cell-derived neurotrophic factor, IGF-1, and VEGF (11–13) or RNAi molecules by the delivery of viral vectors (14–16) to silence SOD1 mutant gene expression. In gene therapy the primary cause of toxicity (i.e. mutant SOD1 proteins) is targeted, unlike drug therapy which usually acts on cell survival or deleterious pathways [29].

Reduction of myocardial reperfusion injury through an effective immunization with SOD and catalase has also been hypothesised. Indeed, the cardioprotective effect of intracoronary infusion of SOD may further increase with coadministration of catalase. It is proven that calcium antagonists, rennin-angiotensin system antagonists, Na⁺/H⁺ exchanger inhibitors, nitric oxide donors and adenosine induce cardioprotective effects during primary angioplasty for the management of acute myocardial infarction. When these reagents were administered using intracoronary infusion, their efficiency has increased. Another way to attenuate myocardial ischemia-reperfusion injury is anterograde intracoronary and intravenous administration of anti- P-selectin and anti- ICAM-1 antibodies. The ideal injection route for these antibodies is retrograde intracoronary infusion, which has direct access to postcapillary venules [29].

Application of inhibitors of cellular redox maintaining proteins which reduce intracellular ROS is complementary to the use of pro-oxidant molecules, for example, administration of catalase or SOD in association with various peroxidases. 2-Methoxyestradiol by increasing cellular ROS formation due to its inhibition of SOD, enhances the cytotoxic effects of apoptotic agents and displays anti-leukemic activity in culture. On the other hand it has been hypothesized, continuous mitochondrial ROS formation leading to oxidative stress and mitochondrial damage has link to degenerative diseases and aging. Based on the ROS etiology of aging the

ROS inhibition should have therapeutic benefit. Administration of antioxidants manganese (III) tetrakis (4-benzoic acid) porphyrin (MnTBAP) or N-acetylcysteine also improved glucose homeostasis and insulin sensitivity in obese insulin-resistant mice [31]. MitoQ, a coenzyme Q analog is currently in trial for the treatment of Parkinson's Disease due to its potential mitochondrial ROS inhibition. Knowing the beneficial effects of ROS shouldn't underscore the importance of a detailed knowledge of pathological conditions under which ROS formation is happening, as well as the identity and biological half-life of the ROS produced.

Free radicals are generally involved in many pathological processes. The injuring mechanism of reactive radical species is concentration dependent, which finally damages all cellular constituents. Any insufficiency or functional failure in the body antioxidant systems results in the shortening of the lifespan. Therefore, the first therapeutic approach is restoring the normal function of the antioxidant enzymes like SOD.

4.4. Mitochondrial K_{ATP} channels target for therapy

Potassium channel openers (KCOs) are agents, discovered in the early 1980s, that act by stimulating ion flux through K^+ channels. Many drugs such as, diazoxide, nicorandil, and cromakalim have been identified as KCOs. KCOs act on two types of ion channels: Ca^{2+} activated K^+ channels (BK channels) and ATP-regulated K^+ channels (K_{ATP} channels). KCOs were first identified by their antihypertensive or antianginal mode of action. Now, they are at various stages of development as and cardioprotective agents. Preclinical and clinical evidence also supports the therapeutic role of KCOs in vascular and pulmonary hypertension, and the treatment of overactive bladder. Until recently, it was believed that the effects of KCOs were entirely attributed to the modulation of K^+ channels in cell plasma membranes. However, it is now proven, that new targets for KCOs exist in intracellular membranes including those of mitochondria, zymogen granules, and sarcoplasmic reticulum. It seems that Mitochondria are particularly very important targets for KCOs, because the interaction of these compounds with mitochondria appears to mediate the cardioprotection of KCOs. The protective role of mitochondrial ion channels was recently summarized and mitochondrial targets for anti-ischemic drugs were recently described [32].

4.4.1. Potassium channel openers and mitochondrial K^+ channels

A small-conductance potassium channel, with properties similar to those of the K_{ATP} channel from the plasma membrane, in the inner membrane of rat heart and liver mitochondria and designated the $mitoK_{ATP}$. The $mitoK_{ATP}$ channel was blocked not only by ATP, but also, similarly to the plasma membrane K_{ATP} channel, by antidiabetic sulfonylureas. These observations raised the question whether the $mitoK_{ATP}$ channel could be activated by KCOs. In fact, an increased influx of K^+ and depolarization of liver mitochondria in the presence of KCOs was observed. Also, other KCOs were shown to activate potassium ion transport into mitochondria. KCOs such as levcromakalim, cromakalim, and pinacidil have been shown to depolarize cardiac mitochondria. KCO-induced membrane depolarization was associated with an increase in the rate of mitochondrial respiration and decreased ATP synthesis. Moreover, KCOs released cytochrome c and calcium ions from cardiac mitochondria. Despite the

effect on K^+ transport, diazoxide also exhibits a direct effect on mitochondrial energy metabolism by inhibition of respiratory chain complex II in liver mitochondria. Recently, mitoK_{ATP} channel opener BMS-191095 with no peripheral vasodilator activity was described. Using isolated mitochondria or proteoliposomes reconstituted with partly purified mitoK_{ATP} channel and measuring potassium flux demonstrated that heart and liver mitochondrial K_{ATP} channels have some pharmacological similarities with the cell membrane K_{ATP} channel, i.e., both channels are activated by KCOs. Mitochondrial K_{ATP} channels are 1000 times more sensitive to diazoxide than that of cell membrane K_{ATP} channels. This document concluded that the interaction of mitochondrial K_{ATP} channels with KCOs plays a key role in cardioprotection [32].

4.4.2. Mitochondrial K_{ATP} channel: A novel target for cardioprotection

Mitochondrial K_{ATP} channel: A Novel target for Cardioprotection. KCOs mimic hypoxic/ischemic preconditioning in the absence of ischemia in the heart myocardial cells, the reason why antagonists of K_{ATP} channel, like 5-hydroxydecanoic acid and glibenclamide, ameliorate the positive effects of short time hypoxic/ischemic conditions on the heart myocardium. The primary postulation to justify these events includes cell membrane K_{ATP} channels. Newly, it was shown that in fact KCOs including diazoxide affect the mitoK_{ATP} channel in mitochondria. In a complementary approach, it was shown that diazoxide did not activate plasma membrane K_{ATP} channels, but induced oxidation of mitochondrial flavoproteins, due to the activation of mitoK_{ATP} channel. These findings established the fact that the target for the diazoxide protective effects in heart myocytes is the mitochondrial K_{ATP} channel rather than the cell membrane K_{ATP} channel. It is also note worthy that evidence for mitochondrial K_{ATP} channels as effectors of cardiac myocardial preconditioning has also been proven in human subjects. The initial observations on the cardioprotective action of KCOs on mitochondria were further confirmed and developed in a series of reports. It has been shown that other KCOs such as nicorandil, cromakalim, and pinacidil modulate mitochondrial Ca²⁺ uptake, respiration, mitochondrial membrane potential, ATP generation, and mitochondrial Ca²⁺ uptake. The main question remains how the opening of the mitoK_{ATP} channel could protect cells against ischemic damage. 1) Opening of the mitoK_{ATP} channel followed by mitochondrial swelling could improve mitochondrial ATP handling and/or production. 2) The protective effect of mitoK_{ATP} activation could be mediated by lowering Ca²⁺ overloading of mitochondria. In fact, it was found that diazoxide preserves mitochondrial function in ischaemic rat cardiomyocyte. It is now proven that hypoxia approximately decreases mitochondrial oxygen consumption rate to 40% of the normal value, and administration of diazoxide maintains the prehypoxic mitochondrial oxygen consumption rate during hypoxia/ischemia. Cardiac ATP concentration was significantly raised following diazoxide treatment. Secondly, by lowering Ca²⁺ overloading of mitochondria the protective effect of mitochondrial K_{ATP} activation could be induced. It was shown that the opening of the mitochondrial K_{ATP} channel may increase mitochondrial reactive oxygen species (ROS) formation. This increase could lead to protein kinase C activation, which is known to be necessary for the cardioprotection. Besides, it seems that mitochondrial K_{ATP} channel is enrolled in delayed preconditioning because of an alteration in expression of "protective" proteins (3). It was that pretreatment of hippocampal neurons with

cromakalim and diazoxide increases the expression level of Bcl-2 and Bcl-XL which are involved in the control of apoptosis Bcl-2 [32].

5. Mitochondria as a biosensor for drug development

Extensive study over the last 50 years indicates that many medications can induce mitochondrial damage [33]. Medication-induced dysfunctions include the alteration of mitochondrial components and metabolic pathways. These dysfunctions are a major challenge and problem for drug development. There is mounting evidence of the mitotoxicity (table 2).

Interestingly knowledge of the mechanisms that trigger drug-induced mitochondrial damage will be helpful in the development of strategies to decrease the potentially toxic effects of medications. Additionally, these issues affect the most aerobically poised organs such as heart and kidneys or organs exposed to higher concentrations of the drug for example liver. Recently using mitochondria as a biosensor for determination safety of drug development has increased. The reasons are as follows: A) in general, mitochondria control many of the pro-death and anti-death cell signals; B) a number of reports describe an association between patients receiving medication and effects on mitochondrial metabolism 3) drug safety has become a priority of many pharmaceutical companies [4].

It is quite obvious that mitochondria are key elements of cell life which several well known drugs induce toxic effects on them in several non-target and target organs. As soon as possible by improvement of preliminary drug safety assessment the possibility of drug toxic reactions during clinical practice will be avoided. Depending on the targeted organ, severe *in vitro* mitochondrial impairment may be sufficient to ban an efficient drug in the market or preventing a promising drug candidate from further clinical trials. Drug companies now have a new dilemma, which is to realize how much of the evaluated mitochondrial toxicity is a key predictor of the drug pharmacological or adverse effects. Pharmaceutical suppliers have also now a difficult problem which is to know how much of the supposedly mitochondrial impairment is a component of the therapeutic effect. On the other hand, it may be a tough choice to remove dispensing drugs showing a certain degree of mitochondrial toxicity *in vitro* evaluations but with a very unique significant therapeutic effect. Despite showing mitochondrial toxicity, sometimes pharmaceutical companies may decide to push the lead candidate molecule forward for further *in vivo* assays in order to also clarify ways of reducing adverse mitochondrial toxicity [3]. Some pharmacological strategies could be used to decrease mitochondrial toxicity. For example in the cardiac toxicity of doxorubicin (DOX), One possibility is to improve drug targeting, decreasing the amount of drug that reach non-target organs. One successful example of this strategy is the use of pegylated liposomal DOX, which has a quite different pharmacokinetic profile including an increased circulation time and a decreased volume of distribution [34]. Another strategy is the co-administration of protective agents, one example of which being the preventive role of the beta-blocker carvedilol on DOX induced cardiac mitochondrial impairment.

Hepatic Toxicity	Renal Toxicity	Cardiovascular Toxicity
- Metformin	- Cysplatin	- Lidocaine
- Valproic acid	- Gentamicin	- Doxorubicin (DOX)
- Ketoconazole	- Doxorubicin (DOX)	- Celecoxib
- Isoniazid	- Cyclosporin A	- Sorafenib
- Nefazodone	- Statins	- Bupivacaine
- Lamivudine	- Ifosfamide	- Diclofenac
- Divalproex Sodium	- Tenofovi	- Daunorubicin
- Tenofovir		- Ibuprofen
- Didanosine		- Piroxicam
- Tamoxifen		- Thiazolidinediones
- Nevirapine		- Indomethacin
- Zidovudine (AZT)		- Rosiglitazone
- Stavudine		- Atenolol
- Flutamide		-Nucleoside reverse transcriptase inhibitors (NRTIs)
- Abacavir		- Meloxicam
- Phenoformin		- Pioglitazone
- Zalcitabine		- Sulindac
		- Zidovudine (AZT)
		- Idarubicin
		- Piroxicam
		- Mefenamic acid
		- Daunorubicin

Table 2. Examples of Drugs with Black Box Warnings for Mitochondrial Toxicity

Refining of the different methodologies results into higher achievement in the isolation of functional mitochondria from different organs, which can be used in further mechanistic studies to identify tissue-specific drug-induced mitochondrial toxicity. Nevertheless, studies with isolated mitochondria lack the complexity associated with experiments in intact cells, isolated organs or even in vivo studies. But the use of isolated mitochondrial fractions helps determining precise sites of action of the molecule on mitochondria. If everything works OK, the accurate drug safety assessment would correlate data in isolated mitochondria with data collected in intact cells and *in vivo* (figure 3). One important issue in this discussion includes what can be gained by using mitochondria as a biosensor to search drug safety. One particular example was nefazodone, an anti-depressant. This drug was withdrawn from the market in 2004, following several clinical reports of serious liver toxicity [35]. Would the use of mitochondria as cost effective accelerated biosensors of drug safety helped in avoiding the entry of the drug in the market? The answer is, maybe so. Dykens *et al.* demonstrated that nefazodone is highly toxic to isolated liver mitochondria, human hepatocytes and HepG2 cells, showing an obvious cytotoxicity even when cell lines were used. [36] If an initial assessment of mitochondrial toxicity for nefazodone had been done, it is very unlikely that the drug would have been pushed forward for further clinical trials.

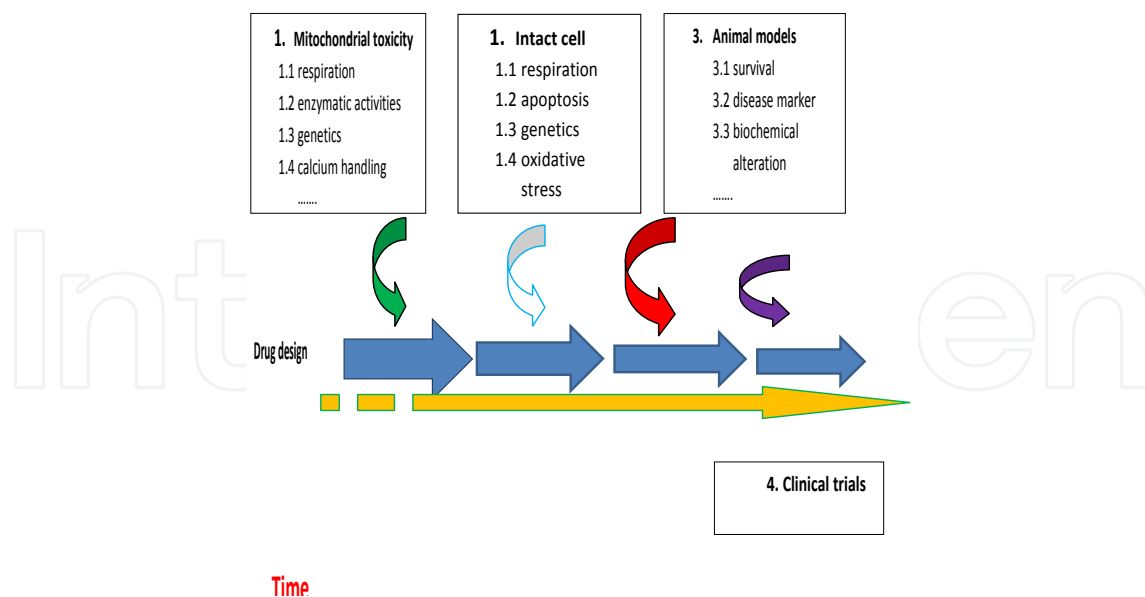


Figure 3. Basic scheme of the drug development process involving mitochondria as an important marker for drug-induced toxicity. The thickness of each arrow exemplifies the number of different molecules in each evaluation stage. Drug toxicity on mitochondria is proposed as the bottleneck step in decision-making [3].

It is now apparent that mitochondrial toxicology has become an area of interest to the industry, since a primary assessment of mitochondrial toxicity of a range of compounds can be performed in a fast and relatively inexpensive way, avoiding some later human toxicity problems that may arise during subsequent testing stages or even during clinical use. Some companies will focus more on investigating direct drug-induced mitochondrial dysfunction, others will rather measure drug-induced alterations in mitochondrial-relevant genes. Whatever the chosen strategy is, the final outcome is the prediction of drug safety based on a mitochondrial end-point.

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