The role of mitochondria in pharmacotoxicology: a reevaluation of an old, newly emerging topic

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Mitochondria can represent a primary or secondary drug target (102, 112). However, some aspects of drug-mitochondria interactions may still be underestimated because of the difficulty in foreseeing and understanding all potential implications of the complex pathophysiology of mitochondria. Insufficient consideration of mitochondrial pharmacotoxicology may also be due to a lack of knowledge about acquired mitochondrial diseases, which are a heterogeneous and growing class of disorders ranging from Type 2 diabetes to neurodegenerative diseases and cancer (30, 32, 105).

A pathogenetic role for mitochondrial dysfunction has been invoked in a vast number of illnesses without sufficient experimental support to precisely establish the molecular pathophysiological mechanisms. Indeed, mitochondrial physiology and pathophysiology are very complex, and the role of the organelles in bioenergetics is strictly linked to other essential functions such as anabolic pathways, redox balance, cell death and differentiation, and mitosis, along with more specialized cell functions including calcium homeostasis and thermogenesis, reactive oxygen species (ROS) and reactive nitric oxide species signaling, ion channels, and metabolite transporters. The same complexity and heterogeneity can be surmised from the range of congenital mitochondrial diseases, providing further evidence of the difficulty in correctly approaching mitochondrial pathophysiology.

These unique aspects of mitochondria should stimulate us to pay more attention than that usually devoted to both the toxic and therapeutic aspects of the interrelationships between drugs and mitochondria. Moreover, their typical structural and functional characteristics may make mitochondria a valuable target for xenobiotics (30, 32).

Mitochondria: Structure-Function and Pharmacotoxicology Relationships

Two main structural and functional aspects of mitochondria should first be considered: the presence of different organelle subcompartments in mitochondria and the fact that mitochondria have their own DNA. Structurally, mitochondria are very diverse across organs and tissues, but all mitochondria contain two lipid bilayer membranes. The outer membrane delineates the organelle and is structurally similar to other cell membranes, being rich in cholesterol and permeable to ions. The
Mitochondria play a critical role in supplying the cell with the bulk of its ATP needs via oxidative phosphorylation (oxygen); thus any cell type or tissue with a high aerobic energy requirement is more likely to be affected when this organelle is dysfunctional. In addition, the enzymes necessary for several specialized metabolic processes (fatty acid β-oxidation, urea synthesis, heme synthesis) reside within the mitochondrial matrix. As a result, tissues that rely heavily on these processes are also frequent targets of mitochondrial toxins. For these reasons there are numerous common syndromes associated with mitochondrial toxicity including lactic acidosis, cardiac and skeletal myopathy, peripheral, central, and optic neuropathy, retinopathy, ototoxicity, enteropathy, pancreatitis, diabetes, hepatic steatosis, and hemotoxicity. Combinations of these effects (or different manifestations of toxicity in different individuals treated with the same compound) are not uncommon and are strong indicators that the underlying toxic insult involves mitochondria. Mitochondrial toxicities in general tend to be chronic injuries with somewhat variable manifestations. Most cells contain a large number of mitochondria that allow for some functional reserve, and cellular injury or dysfunction will occur only when enough mitochondria are irreparably damaged and the cell cannot meet its energy demands. When cells divide, the mitochondria apportionment between them is random (“heteroplasmy”): one daughter cell may contain primarily normal mitochondria while the other gets a dispropor-
tionate share of damaged mitochondria, resulting in a patchy distribution of damaged cells within a tissue.

**Mitochondrial DNA.** mtDNA may be damaged by drugs through different mechanisms. A well-known exogenous agent capable of oxidatively damaging mtDNA is ethanol (26). Some drugs can selectively damage mtDNA by inhibiting its synthesis, as has been recently exploited with the introduction of nucleotide reverse transcriptase inhibitors (NRTIs). These compounds are nucleoside analogs that are taken up by cells and sequentially phosphorylated to the active triphosphate form (22, 66). The nucleotide triphosphates can thus be used as substrates by retroviral reverse transcriptase, while their incorporation into the nascent DNA chain results in chain termination. The triphosphate forms of these analogs have also been shown to be potential substrates for pol-γ, the unique mtDNA polymerase, and can similarly result in chain termination during mtDNA replication (22). Additional effects on mtDNA synthesis result from the fact that the conversion of the monophosphorylated to the triphosphorylated form is extremely inefficient within mitochondria. Consequently, these monophosphorylated forms can build up to high (mM) levels in the mitochondrial matrix and at such high levels can have other effects on mtDNA synthesis. These include inhibition of the exonuclease function of pol-γ (resulting in decreased replication fidelity) and also, as recently shown with zidovudine, may significantly inhibit thymidine phosphorylation, thus affecting DNA replication by depletion of a necessary substrate (111). This DNA pol-γ dysfunction, which induces a progressive depletion of mtDNA, ultimately interferes with the synthesis of essential proteins of the mitochondrial respiratory chain (MRC) (65, 72). The consequent disruption of the electron respiratory chain results in reduced ATP synthesis and electron leakage, leading to increased production of free radical species. Enzyme assay and cell culture studies of NRTIs have demonstrated the following hierarchy of mtDNA pol-γ inhibition: zalcitabine > didanosine > stavudine > lamivudine > zidovudine > abacavir (96). In vitro investigations have also documented impairment of mitochondrial adenylate kinase and the adenosine diphosphate/adenosine triphosphate translocator. Inhibition of pol-γ and other mitochondrial enzymes can gradually lead to critical mitochondrial dysfunction and cytotoxicity. The clinical manifestations of NRTI-induced mitochondrial toxicity resemble those of inherited mitochondrial diseases, i.e., hepatocellular steatosis, lactic acidosis, myopathy, peripheral neuropathy, and, intriguingly, nephrotoxicity. Fat redistribution syndrome, or human immunodeficiency virus (HIV)-associated lipodystrophy, is another side effect attributed to NRTI therapy (18). The morphological and metabolic complications of this syndrome are similar to those of the mitochondrial disorder known as multiple symmetric lipomatosis, suggesting that this too may be related to mitochondrial toxicity (59, 65, 117).

**Mitochondrial respiratory chain.** Drug-induced derangements of the MRC can occur at any of the four protein complexes in the respiratory chain. Effects on complex IV (cytochrome-c oxidase), however, are the most severe because this is the step where oxygen is reduced to water. Inhibition of complex III can also frequently result in the generation of ROS as a consequence of the intrinsic characteristics of the electron transfer process to this complex from reduced ubiquinone.

With respect to iatrogenic mitochondrialopathies, many molecules are well-known inhibitors of mitochondrial complexes. Some of these toxic compounds (including rotenone, antimycin, cyanide, oligomycin, and mixothiazol) have been widely employed to analyze the function of the mitochondrial bioenergetic machinery in general and of electron transport in particular, and have already been the subject of numerous exhaustive reviews and books (33, 40, 50, 52). Interactions of drugs of clinical interest for the mitochondrial electron respiratory chain have not been as well studied. Nevertheless, several drugs are known to act by partially inhibiting, directly and/or indirectly through their metabolites, components of the MRC; examples of such drugs include amiodarone, perhexiline, flutamide, and anthralin (26, 35, 42, 43). The molecular mechanisms by which these drugs impair the MRC have not been resolved. Two hypotheses have been postulated: 1) a direct inhibition of a protein subunit of one or more enzyme complexes and 2) an electron diversion from the MRC by drugs that act as spurious acceptors (60).

Strong support for the importance of these iatrogenic mitochondrialopathies has been provided by a chemically induced parkinsonism resulting from accidental poisoning from 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). The condition is produced as an impurity in batches of the illegally made “synthetic heroin” 1-methyl-4-phenyl-4-propionoxyppiperidine (MPPP). In particular, this by-product, via its metabolite MPP⁺, seems to mainly affect complex I of the mitochondrial oxphos pathway preferentially in dopaminergic neurons (61). Importantly, experimental and clinical data showed that such iatrogenic parkinsonism is characterized by the same neuropathological features as the idiopathic forms (i.e., loss of substantia nigra dopaminergic neurons, Lewy bodies, and typical accumulation of neuromelanin in microglia and extracellular matrix) (34).

There are other molecules that are usually considered real complex I inhibitors, including some well-established drugs (papaverine, meperidine, cinnarizine, amytal, haloperidol, and ketoconazole and its analogs) (38, 78, 98, 101, 109). These molecules share a common structural motif, a cyclic head and a hydrophobic tail (25, 31, 32, 74). Intriguingly, this typical structure is also present in another class of therapeutic agents, the so-called fibrates (clofibrate acid, bezafibrate, gemfibrozil) and in some of their derivatives, the thiazolidinediones (ciglitazone, troglitazone, pioglitazone). Our studies (90, 92), recently confirmed by Brunmair et al. (11, 12), showed that these compounds can also inhibit mitochondrial complex I, resulting in the metabolic consequences typical of their pharmacological activities (hypolipidemic and hypoglycemic effects); such inhibition may explain some of their toxic effects (rhabdomyolysis, acute liver failure) that intriguingly resemble those of inherited mitochondrialopathies. More than 60 types of compounds are well-known inhibitors of complex I, and this number continues to grow. This tendency of xenobiotics to inhibit complex I (mitochondrial NADH:ubiquinone oxidoreductase; EC 1.6.5.3) may depend on the intricate structure of this enzymatic complex, which consists of at least 40 different polypeptides strongly embedded in the inner mitochondrial membrane. This unique feature explains the mitochondrion’s great vulnerability to lipophilic molecules (25, 30, 74). Regarding potential toxicity, complex I is a secondary target of nitric oxide (NO) in general and of nitrogen radical
species in particular; this should be kept in mind when patients are being treated with old and particularly new NO donor drugs (10, 15, 76, 85, 89).

Complex II of the electron respiratory chain, succinate dehydrogenase (SDH), is less commonly studied in mitochondrial pharmacotoxicology, which is surprising considering that it also plays a role in the tricarboxylic acid cycle. Apart from the common inhibitors usually employed in experimental studies (i.e., malonate, carboxin, 3-nitropitonionic acid), it is worth pointing out that some cis-crotonalide fungicides, diazoxide, and, more recently, some fluoroquinolones, chloramphenicol succinate, and anthracycline drugs are complex II inhibitors that also inhibit other mitochondrial components (102, 112). Importantly, recent reports regarding the role of SDH (or of one of its components: the B, C, and D subunits) in tumor susceptibility have opened up a new perspective in research on the modulation of oncosepression and/or oncopromotion by mitochondria (4, 8, 45, 83, 95). The mechanism of this tumor promotion by SDH and by fumarate hydratase (FH) has been ascribed to an intriguing metabolic signaling pathway that starts with the physiological substrates of these enzymes (i.e., succinate and fumarate, respectively). In fact, these metabolites accumulate in mitochondria because of the inactivation and/or low activity of SDH and FH, leak out to the cytosol, and there inhibit a family of prolyl hydroxylase enzymes. This inhibition, in turn, may render neoplastic cells more resistant to apoptotic signals and activate a pseudohypoxic response (mediated by hypoxia-inducible factor) that enhances glycolysis (84). Other signals and activate a pseudohypoxic response (mediated by succinate and fumarate, respectively). In fact, these metabolites contribute to nuclear DNA damage, mutagenesis, and ultimately tumorigenesis (58). Considering the potential value of these data in terms of pathophysiology and pharmacotoxicology, together with recent publications on a role of ROS as tumor suppressors and senescence inducers (104), elucidation of the prevalent/causative molecular mechanisms at the basis of SDH/FH tumor suppressor/promoter activities may provide better insight into the link between cancer and mitochondria, and ultimately carcinogenesis.

For complex III (ubiquinol: cytochrome c oxidoreductase; EC 1.10.2.2), there are likewise a large number of inhibitors acting at different levels. Among these we found myxothiazol and antimycin A, which up to now have not been shown to have clinical value (102, 111).

On the contrary, there are well-known morbid entities based on complex IV (cytochrome-c oxidase) inhibition, such as cyanide and hydroxylfide poisoning and carbon monoxide intoxication. More important, although not yet fully defined in all their implications, are the effects arising from not only the interaction of NO and peroxynitrite (ONOO−) with cytochrome-c oxidase, but also their interaction with all the other mitochondrial components. NO mainly interacts at physiological levels with cytochrome-c oxidase, leading to a competitive and reversible inhibition of its enzymatic activity (10), promoting alterations in the electrochemical gradient that could affect calcium uptake, and regulating processes such as mitochondrial transition pore opening and release of prosapotic proteins (53). A direct effect of NO on the permeability transition pore complex is generally accepted (37). Moreover, large or persistent levels of NO in mitochondria could also promote the formation of mitochondrial oxidants like ONOO−, either extra- or intramitochondrially, leading to oxidative damage, most notably of complexes I and II of the electron transport chain but also of ATPase, aconitase, and Mn-superoxide dismutase (10, 76, 85). It is important that the preceding information be taken into account during pharmacological treatment with new and old NO donors, because their kinetic NO release is rather difficult to finely regulate, and therefore abrupt changes in concentration may eventually lead to abnormally high NO levels (10, 15, 85). Inhibition of complex IV by local anesthetics, such as dibucaine and lidocaine, appears less important from a clinical viewpoint, although this inhibition shows an interesting positive correlation with the degree of lipophilicity of the molecule (30, 60).

With respect to interactions between drugs and mobile electron carriers, some polycationic molecules can lead to dysfunction that can alter interactions of biomembranes and/or cytochrome oxidase with cytochrome c (14, 56, 119). In this respect, a recent (early 2001), dramatic toxic effect related to cerivastatin was characterized by severe episodes of rhabdomyolysis and secondary acute renal failure due to the introduction on the market of a new high-dosage formulation of this statin, administered alone or in association with fibrates. This 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor also reduces the endogenous synthesis of coenzyme Q (a disregarded aspect), which may promote mitochondrial dysfunction affecting both complex I and II in predisposed patients (i.e., patients with bioenergetic mitochondrial derangement for concomitant diseases) with concurrent toxic pharmacological treatment (for example, with fibrates or for slow drug inactivation or other pharmacogenomic causes) (24, 41, 93).

A particular class of agents capable of interfering with the MRC have been proposed to act as alternate electron acceptors by extracting electrons from intermediates in the respiratory chain in competition with their natural substrates. These substances may also alter the redox cycle, passing electrons back to the respiratory chain at a later point, thus bypassing sites in the chain that are essential for energy generation. Compounds that frequently do so are the quinones such as doxorubicin, menadione, and paraquat. In particular, menadione (vitamin K3) is a well-known electron respiratory chain uncoupler that shunts electrons from complex I directly to complex IV, and its toxicity depends on free radical production along with a dramatic depletion of ATP. Doxorubicin, a potent broad-spectrum antineoplastic agent, is characterized by an intriguing myocardotoxicity that seems to depend, at least in part, on its ability to alter the redox cycle of the MRC by interacting with some of its molecular components. Briefly, doxorubicin, or one of its metabolites, can accept one electron from complex I, generating a highly unstable semiquinone free radical intermediate that, in turn, can undergo three possible fates: 1) reduction to the corresponding hydroquinone, 2) formation of covalent adducts that interact with DNA and proteins, or 3) transfer of the unpaired electron to other acceptors (glutathione, thiol groups, heme proteins, tocopherols, or ascorbic acid and/or directly to oxygen) (102, 113).

Importantly, many of the drugs capable of deranging the MRC also induce reactive intermediates, generated by mitochondrion-specific processes, that are often considered the
effectors responsible for cellular and/or molecular damage. There are also xenobiotics that are capable of inducing ROS generation without directly deranging the MRC, examples of which are haloalkenyl cysteine conjugates such as hexachlorobutadiene (which form reactive thiolos subsequent to their activation by the mitochondrial enzyme β-lyase), 4-thiaalkanotes (activated by fatty acid β-oxidase), and valproic acid (activated by acyl-CoA synthase) (39, 106).

Oxidative phosphorylation. Compounds that dissipate the proton gradient between the intramembrane space and the matrix interact at the oxphos level. They can act as direct protonophores, shuffling hydrogen ions into the matrix (2,4-dinitrophenol is the classic example), or as ionophores, exchanging hydrogen ions for other mono- or divalent cations, or may generally increase the permeability of the inner membrane. The dissipation of the proton gradient without ATP generation can result in the generation of heat, and, in extreme conditions, a malignant hyperthermia syndrome can occur (32, 33). In this regard we can include most of the nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin, diclofenac, and nimesulide [importantly, the pathogenesis of NSAID enteropathy also involves the uncoupling of mitochondrial oxphos, which alters the intercellular junction and increases intestinal permeability with consequent intestinal damage (63)], but also some antitumor drugs and antipsychotic, hypolipidemic, and antimycotic compounds. Interestingly, for all these drugs, the main structure-activity relationship is characteristically based on a lipophilic weak acid group (40, 77, 112).

Other xenobiotics may interfere with oxphos by direct inhibition of ATP synthase. The majority of these molecules are mycotoxins (such as oligomycin), but there are also well-known drugs such as propanolol, local anesthetics, and diethyldithiole in. Intriguingly, some pharmacological activities of propanolol should be carefully reevaluated, especially with regard to their typical side effects such as cardiac output reduction.

Mitochondrial metabolic processes. Xenobiotics can interfere with catabolic and anabolic pathways in mitochondria. We described above some drugs that can induce dysfunction of the tricarboxylic acid cycle at the SDH and FH sites. In light of the etiopathogenic role of the congenital enzymopathic counterparts in serious forms of neurodegenerative diseases and cancer, the potential toxicological value of this dysfunction should not be underestimated.

In this respect, recent evidence reported by Nulton-Persson et al. (80) has shown that treatment with salicylic acid, and to a lesser extent, acetylsalicylate, increases the rate of uncoupled respiration in isolated cardiac mitochondria, in agreement with previous data (77). However, under the experimental conditions employed, loss of state 3 respiration resulted from inhibition of the tricarboxylic acid cycle enzyme α-ketoglutarate dehydrogenase. In particular, a kinetic analysis indicated that salicylic acid acts as a competitive inhibitor at the level of the α-ketoglutarate binding site. In contrast, acetylsalicylate inhibited the enzyme in a noncompetitive fashion, consistent with its interaction with the α-ketoglutarate binding site followed by enzyme-catalyzed acetylation. Furthermore, it was recently observed that cyclosporin reduced the concentrations of tricarboxylic acid cycle intermediates and inhibited mitochondrial oxphos at the level of ATP synthase in a time-dependent fashion (21). The real mechanism of such metabolic dysfunction is still being debated (i.e., energetic failure, reduced protein synthesis, or real enzymatic inhibition) (21). At first presentation, isoniazid overdosage can easily be mistaken for a case of diabetic ketoacidosis. Although the exact mechanism is still controversial, the inhibition of pyruvate conversion to lactate and the interference with NADH synthesis in the tricarboxylic acid cycle have both been suggested to contribute to the lactic acidosis observed in isoniazid toxicity. Intriguingly, serum isoniazid levels have not been helpful in the evaluation of isoniazid toxicity and treatment (1).

Another fundamental metabolic pathway that is often affected by xenobiotics is β-oxidation. Many drugs (tetracycline derivatives, NSAIDs such as ibuprofen and irprofen, glucocorticoids, antidepressants such as aminepentine and tianeptine, some statins, fibrates, estrogens, and some antiarrhythmics and antiangiual drugs such as amiodarone and perhexiline) can directly and/or indirectly interfere with mitochondrial fatty acid oxidation, with important safety concerns, particularly with respect to the liver. However, the precise molecular mechanisms underlying this dysfunction have not been clearly established. The pathogenesis often appears secondary to an MRC derangement that heavily hampers NADH and/or FADH₂ oxidation (26, 32, 42). With respect to fibrates and thiazolidinediones, it is interesting to note that our data (90, 91), confirmed by Brunmair et al. (11, 12), showed that the dysfunction in glucose metabolism and/or β-oxidation significantly correlates with the level of complex I inhibition. Interestingly, Vickers et al. (110) recently showed a direct inhibition by etomoxir of the mitochondrial β-oxidation rate-limiting enzyme carnitine palmitoyltransferase I, which is associated with oxidative stress, inflammation, and apoptosis in the liver.

Mitochondrial protein synthesis. The close similarity between bacterial and mitochondrial ribosomes makes the latter a potential target for bacteriostatic antibiotics such as chloramphenicol, aminoglycosides, tetracycline, and the newest family, the oxazolidinones (73). For the latter class, a direct correlation has been demonstrated in both clinical and toxicological studies between the bacterial MIC₉₀, the IC₅₀ for mitochondrial protein synthesis, and the potential for mammalian toxicity, suggesting that this toxicity is manifested as a consequence of their effects on mitochondria (73, 112). In the four years since FDA approval of the oxazolidinone antibiotic linezolid, a number of papers have surfaced reporting lactic acidosis, peripheral and optic neuropathy, thrombocytopenia, and pure red cell aplasia resulting from prolonged use, which are all syndromes commonly associated with mitochondrial injury (73). It is worth noting that antibiotic inhibition of mammalian mitochondrial protein synthesis is often disregarded by not considering synergistic pharmacological interactions with other mitochondrial toxins.

Mitochondrial channels and mitochondrial permeability transition pores. Many well-known drugs (e.g., potassium channel openers such as nicorandil and diazoxide as well as antidiabetic and antumor sulfonylureas) modify the activity of different mitochondrial channels, which have a fundamental role in maintaining the electrolyte homeostasis of mitochondria. Although these drugs are known to interact with components of various ion channels, the potential toxic effects and/or therapeutic applications of these mitochondrial channel alterations have not yet been clearly defined. Similar considerations should be made for drugs that interact with or modulate...
components of the mitochondrial permeability transition pore (e.g., cyclosporine A binding of cyclophilin D, lonidamine binding of adenine nucleotide translocase, and drugs binding to the mitochondrial benzodiazepine receptor for which the putative toxic effects remain controversial) (6, 13, 23, 81, 37, 49).

The permeability transition pore is a high-conductance, nonspecific pore in the inner mitochondrial membrane composed of proteins that link the inner and outer mitochondrial membranes. When the permeability transition pore is opened as a result of exposure to high calcium or inorganic phosphate, depletion of NAD(P)H, alkaline pH, or ROS, low-molecular-weight substrates can freely penetrate the mitochondrial matrix, carrying along with them water and resulting in mitochondrial swelling and the release of cytochrome c into the cytosol. Cytochrome c release triggers a cascade of events that will lead to either apoptosis (in ATP-replete cells) or necrosis (in ATP-depleted cells). The toxicity of t-butyl-hydroperoxide and valproic acid and the chronic hepatotoxicity of diclofenac and other NSAIDs are mediated by this mechanism (8, 86). In general, the opening of this particular mitochondrial pore represents the common final event produced by numerous cell and mitochondrial toxins.

There are ongoing debates about drug actions and mitochondrial channels. For example, Skalska et al. (99) have proposed that sulfonylureas induce mitochondrial swelling, the lowering of Δψ, and an efflux of calcium from the matrix, mainly by activating the mitochondrial permeability transition. On the other hand, Fernandes et al. (36) hold that sulfonylureas interfere with mitochondrial bioenergetics mainly by permeabilizing the inner mitochondrial membrane to chloride ions and promoting a net chloride-potassium cotransport inside mitochondria.

**Mitochondria and Drugs: Therapeutic Potential**

**Antioxidants.** The first class of drugs developed specifically for the treatment of mitochondrial dysfunction are antioxidants, exemplified by 2,3-dimethoxy-5-methyl-6-decaprenyl-1,4-benzoquinone [coenzyme Q10 (CoQ10)], a fat-soluble quinone with a side chain of 10 isoprenoid units. Apart from its physiological electron carrier function, CoQ10 also seems to stabilize the MRC complexes and acts as a potent scavenger of oxygen free radicals. Accordingly, CoQ10 has been applied therapeutically in different congenital oxphos diseases (19, 64, 88, 120). Interestingly, some positive effects have been also reported in neurodegenerative disorders in general and in Alzheimer disease in particular, although these results have not been confirmed (19, 64, 120). In addition, menadione and phyloquinone (vitamin K compounds, which are well-known uncouplers of the electron respiratory chain) alone or in conjunction with ascorbate have been adopted to treat various congenital oxphos diseases, particularly complex III dysfunction, presumably because their mechanism of action consists of shunting electrons from complex I directly to complex IV (17, 19, 70, 97).

**Mitochondrial channel modulators.** Many mammalian cells have two distinct types of ATP-sensitive potassium (KATP) channels: the classic type in the surface membrane (sKATP) and another type in the mitochondrial inner membrane (mitoKATP). Cardiac mitoKATP channels play a pivotal role in ischemic preconditioning and thus represent a promising drug target. Unfortunately, the molecular structure of mitoKATP channels is not well known, in contrast to sKATP channels, which are composed of a pore-forming subunit (Kir6.1 or Kir6.2) and a sulfonylurea receptor (SUR1, SUR2A, or SUR2B). Recently, it has been observed that some drugs behave as potassium channel openers capable of acting at the level of cellular membranes, including mitochondrial membranes (80). The pharmacological activity of such drugs can therefore also be ascribed to mitochondrial ion modulation. These compounds, which include cromakalim (47), nicorandil (23, 57), and pinacidil (28, 67), were found to modulate K1 channels both in smooth muscle cell membranes and in mitochondria displaying antiapoptotic (nicorandil) or antihypertensive (cromakalim and pinacidil) profiles. Therapeutic activity at the mitochondrial level has also been reported for a variety of older antihypertensive agents, notably diazoxide and minoxidil sulfate, which may also influence the activity of mitoKATP channels (13, 31). Moreover, diazoxide was recently shown to decrease succinate oxidation in a dose-dependent manner (31, 48, 114), albeit at higher concentrations than those necessary to activate mitoKATP. Thus it has been proposed that the cardioprotective effects of diazoxide may result from the inhibition of SDH and a decrease in respiration, rather than from the opening of mitoKATP channels (48). Consistent with the findings that SDH is part of a protein complex capable of transporting K+, SDH may also regulate mitoKATP by means of its physical interaction with the ionophore rather than via its role in oxphos (81).

Such data, once confirmed, could offer new potential therapeutic strategies for different degenerative diseases and stress the potential therapeutic role of mitoKATP modulation.

**Anticancer agents.** Another fundamental pharmacological area in which the interaction between drugs and mitochondria could have striking possibilities is cancer therapy. On one hand, the physicochemical and biological properties of mitochondria expose them to toxic agents, but on the other hand, the same properties could allow us to consider mitochondria as a target of chemotherapeutic agents for selective anticancer therapy (27, 30, 45, 89, 116).

Mitochondrial dysfunction could trigger pathways capable of inducing cell apoptosis or necrosis. Several molecular mechanisms involved in mitochondrial toxicity by different xenobiotics can be and/or have already been utilized in cancer therapy. The following mechanisms are promising mitochondrial therapeutic targets.

mtDNA biogenesis may be affected by inhibiting topoisomerase II (etoposide and analogs, but also cisplatin and 5-fluourouracil and their analogs) or poly-γ (as noted above in the case of antiviral nucleoside analogs) (16, 20, 46, 86, 103).

The electron respiratory chain may be affected by three mechanisms: 1) directly by alteration of a single complex [rotenone and analogs and arsenic trioxide (which partially inhibits) for complex I (2, 94); tamoxifen for complex III and IV (75); and genistein and 17α- or β-estradiol for ATP synthase (3, 71)]; 2) indirectly by generation of free radical species by the same agents that disrupt the electron respiratory chain, by impairing complex I and III (82); and 3) indirectly via photosensitizers or inhibitors of the intrinsic antioxidant defenses of mitochondria (i.e., the superoxide dismutase inhibitor 2-methoxyestradiol) (29).

Mitochondrial permeability transition pores may be affected by interfering with the pores’ physiological functions [i.e.,
Potassium channel opening can be affected by analogs of dequalinium, diazoxide, and amidarone, which increase the permeability of the mitochondrial membrane to protons or potassium and induce a decrease in Δψ, mitochondrial swelling, decrease in ATP synthesis, and release of cytochrome c (54, 87, 108).

Inhibition of Bcl-2/Bcl-X<sub>L</sub> or activation of Bax/Bak by antisense Bcl-2/Bcl-X<sub>L</sub> or by a single-chain antibody can sensitize apoptosis-resistant cancer cells to chemotherapy (5, 62, 118).

Importantly, the latter two classes of anticancer mechanisms represent interesting and innovative therapeutic approaches in which mitochondria are the primary targets. However, these are still in preclinical testing phases, and the speculated applications in cancer and the real therapeutic index must be accurately evaluated. Moreover, independent of the strategy adopted to induce apoptosis and/or necrosis in cancer cells, the best possible targeting of drugs, first to neoplastic cells and then to their mitochondria, must be ensured. As already stated, some authors (8, 79, 116) have suggested that positively charged amphiphatic molecules can be attracted by and penetrate into mitochondria in response to the highly negative membrane potential and even more so in neoplastic cells, which present a more elevated plasma/mitochondrial membrane potential than differentiated cells. Currently, this strategy represents the best compromise to target proapoptotic drugs specifically to cancer cells. However, it should be kept in mind that the in vivo biological environment is extremely variable in cancer; thus patients may be exposed to dangerous and dramatic side effects.

**Mitochondria and Drugs: Perspectives**

The future of mitochondrial pharmacology appears to be headed first toward the development of therapies for glucidic and lipidic metabolism and energy expenditure disorders. Indeed, recent experimental and clinical research has focused on molecular dysfunction at the mitochondrial level for the pathogenesis of some inherited and acquired metabolic diseases (i.e., some mitochondrial forms of non-insulin-dependent diabetes mellitus, metabolic syndrome, hyperlipoproteinemias). These data have been confirmed by experimental evidence showing that it is possible to modulate glucose and/or fatty acid oxidation pharmacologically at the cellular level (32, 79, 102). Modulating the expression and/or activity of the so-called uncoupling proteins in different tissues in general, or of adipose tissue in particular, could represent a new and revolutionary approach to the pharmacological treatment of obesity (51). Interestingly, new and more potent peroxisome proliferator-activated receptor (PPAR) ligands (especially type δ) are being developed for this specific clinical indication that exploit the capability to induce the expression of genes required for fatty acid catabolism and adaptive thermogenesis (51, 115). Given the adverse side effects of some PPAR ligands, an accurate analysis of the potential interactions with mitochondria is imperative (68).

Moreover, recent data indicate that mitochondria in cancer not only represent mere effectors of apoptosis but also have a more complex role in oncogenesis and oncosuppression (30, 44, 86, 90, 91). Additional findings (91, 104) have indicated that electron respiratory chain dysfunction can induce differentiation in different human neoplastic cell lines, suggesting that mitochondria may play additional roles in regulating cell homeostasis. Finally, modulation of the activity of the mitochondrial electron respiratory chain by so-called NO-releasing drugs may expand the potential therapeutic applications in mitochondrial medicine. As these therapies are developed and tested, it may not be important for us to beware of causing undesired toxic effects derived from drug-mitochondria interactions that may not have been carefully considered (76, 89).

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