Mechanisms of Mitochondrial Toxicity (13-Nov-2004)

S. G. Emeigh Hart
Global Safety Assessment (US), AstraZeneca Pharmaceuticals, Wilmington, DE, USA.

Introduction
In addition to their critical role in energy generation in eukaryotic cells, mitochondria are also active participants in a variety of tissue-specific metabolic processes, like urea generation, heme synthesis, and fatty acid β oxidation. They are also structurally, electrochemically and physiologically complex. The result is that there are numerous possible mechanisms by which xenobiotics can interfere with the normal structure or function of this organelle, and that the consequences of this interference are potentially devastating. The ability to predict that a compound is or may be a mitochondrial toxicant is enhanced by an understanding of the unique biological processes of this organelle that predispose it to chemical insult, the common manifestations of mitochondrial malfunction in vivo, and the potential mechanisms by which xenobiotics perturb mitochondria.

Mitochondrial Structure and Physiology
Mitochondria are very structurally diverse, but all mitochondria contain two lipid bilayer membranes. The outer membrane delineates the organelle and is structurally similar to other cell membranes (rich in cholesterol and permeable to ions), but the inner membrane, which isolates the matrix, is virtually devoid of cholesterol, rich in cardiolipin (which binds the proteins of the electron transport chain) and impermeable to ions. This impermeability allows for the maintenance of the electrochemical proton gradient that supplies the energy for ATP generation, and thus the maintenance of the integrity of the inner mitochondrial membrane is critical to mitochondrial function.

The mitochondrion is the only organelle other than the nucleus to contain DNA. The mitochondrial genome consists of a small circular chromosome that contains a total of 37 genes. Thirteen of these encode proteins that are unique components of the electron transport chain. The remaining genes encode 22 tRNAs and two ribosomal RNAs used in the two subunits of the mitochondrial ribosome. The result is that the mitochondrion is fully capable of synthesizing at least some of proteins (the remainder are the products of translocation of nuclear proteins that are synthesized in the cytosol), and its ability to do so is essential to its function in energy generation.

Unlike the nuclear genes, mitochondrial DNA is not protected by histone protein; furthermore, it is located in close proximity to sites where reactive oxygen species are routinely generated, and DNA repair processes are in general less efficient for mitochondrial as opposed to nuclear DNA. The result is that mitochondrial DNA is more likely to undergo mutation than nuclear DNA; the mutation rate is estimated to be at least 10-20 times higher for mitochondrial DNA than for nuclear DNA.

The mitochondrial DNA is able to undergo replication and, to a limited extent, base excision repair. Both of these functions reside in a single DNA polymerase (pol-γ) in mitochondria (as compared to nuclear DNA, which is maintained by at least nine different polymerases). Although pol-γ is a nuclear protein, it has no function other than in mitochondrial DNA replication and thus any mutation or inhibition of this enzyme will be manifest only in mitochondrial DNA.

General Features of Mitochondrial Injury
Mitochondria play a critical role in supplying the cell with the bulk of its ATP needs via oxidative phosphorylation; thus, any cell type or tissue with a high aerobic energy requirement is more likely to be affected when this organelle is dysfunctional.
Additionally, there are several specialized metabolic processes (fatty acid β oxidation, urea synthesis, heme synthesis) whose enzymes reside within the mitochondrial matrix and tissues that rely heavily on these processes are also frequent targets for mitochondrial toxicants. For these reasons, common syndromes associated with mitochondrial toxicity include lactic acidosis, cardiac and skeletal myopathy, peripheral, central and optic neuropathy, retinopathy, ototoxicity, enteropathy, pancreatitis, diabetes, hepatic steatosis, and hematotoxicity. 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 the mitochondria.

Mitochondrial toxicities in general tend to be chronic injuries with somewhat variable and protean manifestations. This is because most cells contain large numbers of mitochondria and there is therefore some functional reserve; only when enough mitochondria are irreparably damaged that the cell cannot meet its energy demands will cellular injury or dysfunction be manifest (the "threshold phenomenon"). In addition, when cells divide the apportionment of the mitochondria between them is random ("heteroplasmy"); one daughter cell may contain primarily normal mitochondria while the other gets a disproportionate share of damaged ones. The result is a patchy distribution of damaged cells within a tissue.

**Common Mechanisms of Mitochondrial Toxicity**

**Inhibition of the Respiratory Chain** - This can occur at any of the four protein complexes in the respiratory chain, although effects on complex IV (cytochrome c oxidase) are the most severe because this is the step where oxygen is reduced to water. Inhibition at complex III can result in the generation of reactive oxygen species as the consequence of the inherent instability of the electron transfer process to this complex from reduced ubiquinone. Classical mitochondrial toxicants (rotenone, cyanide, antimycin) are all inhibitors of the respiratory chain.

**Uncoupling of Oxidative Phosphorylation** - Compounds in this category usually act to dissipate the proton gradient between the intramembrane space and the matrix. They can act as direct protonophores to shuttle hydrogen ions into the matrix (2,4-dinitrophenol is an example), may act as ionophores and exchange hydrogen ions for other mono or divalent cations, or may increase the permeability of the inner membrane in general. The dissipation of the proton gradient without ATP generation can result in the generation of heat and malignant hyperthermia syndrome can result.

**Inhibition of ATP Synthase** - Compounds in this category act directly on the enzyme itself. The majority of these are mycotoxins such as oligomycin.

**Alternate Electron Acceptors** - These are substances capable of extracting electrons from intermediates in the respiratory chain, competing with the natural substrates. These substances may also redox cycle, passing electrons back to the respiratory chain at a later point, bypassing sites in the chain essential for energy generation. Compounds of this type are frequently quinones, such as adriamycin and paraquat.

**Interference with Supply of Educing Substrates** - The function of the electron transport chain is dependent on a steady supply of reducing equivalents in the form of NADH generated by the citric acid cycle. Compounds that inhibit glycolysis, fatty acid oxidation or any of the components of the citric acid cycle will affect mitochondria by this mechanism (examples are fluoroacetic acid and hypoglycin).

**Induction of the Mitochondrial Permeability Transition** - The permeability transition pore is a high conductance, nonspecific pore in the inner mitochondrial membrane that is composed of proteins that link the inner and outer mitochondrial membranes. When opened as a result of exposure to high calcium or inorganic phosphate, depletion of NAD(P)H, alkaline pH, or reactive oxygen species, low molecular weight substrates can freely penetrate the mitochondrial matrix, bringing along water and resulting in mitochondrial swelling and the release of cytochrome c to the cytosol. Cytochrome c release triggers the cascade of events that will lead either to apoptosis (in ATP replete cells) or necrosis (in ATP-depleted cells). The toxicity of t-butyl-hydroperoxide, and the chronic hepatotoxicity of diclofenac and other NSAIDS, are mediated by this mechanism.

**Generation of reactive intermediates by mitochondrial-specific processes** - Examples of these are haloalkenyl cysteine conjugates such as hexachlorobutadiene (which form reactive thiols subsequent to their activation by the mitochondrial enzyme β lyase), 4-thiaalkanoates (activated by fatty acid β oxidase) and valproic acid (activated by acyl-CoA synthase).

**Inhibition of mitochondrial DNA synthesis** - Nucleotide reverse transcriptase inhibitors (NRTI) are the prototypical toxicants
of this class. These compounds are nucleoside analogs that are taken up by cells and sequentially phosphorylated to the triphosphate active form. The nucleotide triphosphates can thus be used as substrates by retroviral reverse transcriptase but 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 γ polymerase, the unique mitochondrial DNA polymerase, and can similarly result in chain termination during mitochondrial DNA replication. Additional effects on mitochondrial DNA synthesis result from the fact that conversion of the monophosphorylated forms to the triphosphates is extremely inefficient within mitochondria; these monophosphorylated forms can build up to high (mM) levels in the mitochondrial matrix and at such high levels can have other effects on mitochondrial DNA synthesis. These include inhibition of the exonuclease function of γ polymerase (resulting in decreased replication fidelity) and also, as has recently been shown for AZT, may significantly inhibit thymidine phosphorylation, thus affecting DNA replication by depletion of a needed substrate.

Inhibition of mitochondrial protein synthesis - The close similarity between bacterial and mitochondrial ribosomes makes the latter a potential target for bacteriostatic antibiotics such as chloramphenicol, aminoglycosides and the newest family, the oxazolidinones. For the latter class, a direct correlation has been demonstrated between the bacterial MIC90, the IC50 for mitochondrial protein synthesis, and the potential for mammalian toxicity seen both in the clinic and in toxicology studies, suggesting this toxicity is manifest as a consequence of effects on mitochondria. In the four years since the FDA approval of the oxazolidinone antibiotic linezolid there have surfaced a number of reports of lactic acidosis, peripheral and optic neuropathy, and pure red cell aplasia resulting from prolonged (>28 days) use, all syndromes commonly associated with mitochondrial injury. In animal studies with linezolid and eperezolid, rapid decreases in reticulocytes that precede the onset of lowgrade anemia, increased myeloid:erythroid ratios and evidence of depletion/maturational arrest in late-stage erythroid precursors have been noted. In the bone marrow of dogs and humans, the presence of increased numbers of "ringed sideroblasts" has been noted, a finding indicating increased intramitochondrial iron accumulation and suggestive of decreased heme synthesis have also been noted following oxazolidinone administration, further suggesting effects on the mitochondrion (the site of the beginning and end stages of heme synthesis). While the enzymes directly responsible for heme synthesis are translocated nuclear gene products, the conversion of iron from the inactive ferric form to the ferrous form required by ferrochelatase occurs during translocation across the inner mitochondrial membrane as the result of the transfer of an electron from Complex V (cytochrome c oxidase) of the respiratory chain; hemoglobin synthesis is thus inexorably linked to the presence of intact mitochondrial electron transport.

References


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