Summary - Mitochondrial Disease and Toxins

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Mitochondrial Disease

The major focus of research and literature has been the study of mitochondrial and nuclear DNA mutations causing a genetically caused mitochondrial disease. This discussion, however, will focus on the non-genetic, or somatogenic, causes of mitochondrial disorders and dysfunction. The symptoms very closely resemble a genetically inherited mitochondrial disease, but the causative agent is acquired by exposure to drugs, environmental or occupational chemicals. Dr. Wallace is a toxicologist, devoted to the study of adverse effects of chemicals on living organisms. Both genetically inherited and acquired mitochondrial disease share a multi-organ presentation, metabolic phenotype (visible or physical presentation), and tend to be progressive over time (slide 2). Toxicity is a term often used to describe acquired mitochondrial disorders.

Genetic Mitochondrial Diseases are caused by mutations to the nuclear (nDNA) and/or mitochondrial DNA (mtDNA) (slide 3). Every cell has hundreds to thousands of mitochondria based on the specific bioenergetic need of the organ. Each mitochondrion has its own circular DNA, which replicates or copies itself throughout its life cycle. Inherited mitochondrial disorders typically arise from mutations in the mitochondrial genome via the X-chromosome given to offspring by the mother or egg. Nuclear genes embedded in the mitochondrial also play a role in inheritance of this disease.

All of the cell's energy, namely ATP, is generated within the five complexes or clusters of up to 42 proteins of the electron transport chain (slide 4). Electrons flow from left to right along the chain. The lower part of the slide depicts the numbers of proteins that each of the five complexes require for normal function and where those complexes are encoded - either in the nDNA or the mtDNA. For example, Complex I utilizes 42 total proteins - 35 which are encoded in the nuclear DNA and the remaining 7 encoded in the mitochondrial DNA. Most proteins are encoded in the nuclear DNA, meaning that if a mutation is acquired in the nuclear DNA that stops the production of the protein, the entire electron transport chain's ability to produce energy becomes dysfunctional.

Somatagenic Etiology of Mitochondrial Disorder - "Mitochondrial Toxicity" describes the etiology of mitochondrial disorders acquired from pharmaceutical, environmental, and/or occupational exposure that damage the cell's ability to produce energy (slide 5-7). MitoAction has two previous guest presentations focusing on drug exposures causing mitochondrial disorders. In 2009, Dr. James Dykens presented "Drug Toxicity and Pharmaceuticals," and in 2010, Dr. Katherine Sims presented "Mitochondrial Toxicity." Toxicity from pharmaceutical sources became clearly evident as medications are more controlled and studied in more depth than environmental and occupational exposures, which are harder to document and quantify. Dr. Wallace has published,"Multiple Targets for Drug-Induced Mitochondrial Toxicity" in 2015.

Chemical Targeting to Mitochondria - after exposure, chemicals and drugs are *not* distributed equally throughout cell (slide 8-12). Certain attributes of the mitochondria tend to draw those chemicals into the mitochondria, creating a disproportionate accumulation of the chemical in the mitochondria compared to the rest of the cell. The more the chemical is present, the more the likelihood of a chemical reaction or adverse effect. Drugs and chemicals with an affinity for Cardiolipin, a specific lipid unique to the mitochondrial membrane, tend to bioconcentrate in this membrane, increasing the likelihood that a reaction will occur, typically an adverse reaction. Secondly, the electrical potential of the membrane targets chemicals to the mitochondria. Within Complex I and IV of the electron transport chain, Hydrogen ions (H+) are pumped from the inside of the mitochondria through the membrane thus separating the positively charged Hydrogen atoms across the membrane, creating a potential or potential energy. This energy is a measure of the capability of those free ions to join in a chemical reaction due to the positive charge of the Hydrogen ions, creating a battery of sorts. Positive charges will want to flow into the mitochondria to reestablish equilibrium, either with the Hydrogen ions or even with other positively charged chemicals. That movement of Hydrogen ions also make the outside of the membrane more acidic and the inside of the membrane more basic, creating yet another gradient. In summary, positively charged chemicals are drawn from the cell contents into and accumulate preferentially in the mitochondria, running the risk of causing dysfunction in energy production. The drugs associated with directly inhibiting each of the five complexes as well as pharmaceutical uncouplers of mitochondrial respiration are listed in detail on slide 11. Chemical (non-drug) inhibitors of the complexes of the electron transport chain and uncouplers of mitochondrial respiration are outlined on slide 12. Pesticides, herbicides, poisons, heavy metals, and more are all toxic to the cell. Note that all the heavy metals listed have two positive charges that are drawn into the mitochondria, interfering with the electrical potentials. These lists are not comprehensive and are still growing.

Substrate Delivery and Mitochondrial Toxicity (slide 13-16) If the substrates needed for the electron transport chain are not available, the cell will "starve," ultimately inhibiting the production of ATP. For example, NADH is a substrates for Complexes I and IV, and Succinate is a substrate for Complex II (slide 14) feeding the electron transport chain. NADH and Succinate are derived from the TCA or Krebs Cycle (slide 15). The TCA cycle can be inhibited by the potent pesticides, Fluoroacetate and

Fluoroacetamide. Working backward, Acetyl CoA, derived from the breakdown of glucose into Pryuvate, is needed for the TCA cycle, but can be inhibited by the chemical, Lodoacetate, again leading to starvation of the mitochondria. The same starvation will happen with fatty acid oxidation when exposed to Valproic Acid and Salicylates (including aspirin) as the Acetyl CoA production needed for the TCA cycle is inhibited. These chemical become indirect inhibitors of mitochondrial respiration by inhibiting substrate delivery.

Molecular Targets for Mitochondrial Toxicity (slides 17-20) from the mitochondrial or nuclear genome to the synthesis of protein incorporated into the mitochondrial structure or electron transport chain, can create chemical-induced mitochondrionopathies.

- Gene Translation - translating the genetic code to a message (messenger RNA)
- Protein Translocation and Assembly - movement of proteins within the cell for assembly within the complexes. A leader segment, or precursor, attaches to the positively charged protein, and therefore, is drawn the mitochondria across the gradient. TOM (Transporter Outer Membrane) and TIM (Transporter Inner Membrane) help to unfold the protein so that one strand at a time is transported to the mitochondria. After diffusion across the mitochondrial membrane from TOM protein complex though TIM protein complexes, the leader segment is clipped off by the protease enzyme, and the remaining protein becomes one of the proteins incorporated into the electron transport chain. Mapping out this process uncovers several potential targets for chemically induced mitochondrial disease due to mutations in the genetic code in the nucleus, cytosolic ribosomes (protein synthesis within the cytoplasm), and/or precursor protein processing. Any chemical that changes the charge of the proteins outside the cell, thus depolarizing membrane potential, will inhibit those proteins from entering the cell as the ion gradient will no longer exist. Drugs that inhibit protease enzymes interfere with the snipping of the precursor from the protein, rendering that compound non functional in the electron transport chain. Most of the 84 proteins needed to produce energy must go through this process through TOM and TIM and are subject to ill effects of toxins along this pathway.
- Mitochondrial DNA Replication and Translation (slide 21-23) begins with a starter sequence, causing the replication of the mitochondrial chain. Topoisomerase enzymes helps to untwist the mtDNA so it can be read, and Polymerase helps assemble individual nucleic acids into a chain. This process is also a target for toxicity and damage.
  - Nucleus Reverse Transcriptase Inhibitors (NRTIs) - drugs designed to inhibit the replication of viral DNA (such as HIV). Human hosts cells have over five DNA polymerase enzymes, but these drugs target just one of these enzymes, namely DNA Polymerase Gamma. The drug is similar to the host nucleotide but is lacking a hydroxyl group, rendering it unable to form chains nor continue synthesis of the DNA chain necessary to replicate. Viral DNA replicated is inhibited when an NRTI is incorporated into the DNA chain in place of the natural state nucleotides.
  - Mitochondria, unfortunately, only have one DNA Polymerase, which happens to also be the DNA Polymerase Gamma. Using these drugs to treat viruses carries the unwanted side effect of inhibiting the replication of the mitochondrial genome. With

time, the cells become deficient in mtDNA, and therefore, cannot make enough of the proteins needed to support energy production.

- Protein Synthesis - making protein from the messenger RNA (slide 24). At the ribosome, the RNA's message is translated into a specific protein and is another target for toxicity. Ribosomes decode RNA into proteins. Nuclear DNA carry many different ribosomes, but mitochondria have only one specific ribosome. Bacteria also carry that same single ribosome because mtDNA was derived from bacteria. Antibiotics are aimed to inhibit that specific ribosome of a bacteria, but can also damage mitochondria. Chloramphenicol, Tetracycline and Linezolid, for example, carry the unwanted side effect of mitochondrial toxicity due to protein synthesis inhibition.

Additional chemicals or environmental mitochondrial toxicities are known to interfere with mitochondrial function, but the mechanism is not yet fully understood (slide 26):

- Cigarette smoke
- Air pollution and particulates
- Poly aromatice hydrocarbons (PAHs)
- Herbicides 2, 4-dichlorophenoxyacetic acide, dinoseb
- mtDNA geneotoxicants, mutagens
- and others

"Hazard" vs "Risk!"

Bear in mind, reports of demonstrated mitochondrial toxicity represent a "hazard" that may or may not be a "real" risk under normal or intended exposure conditions! Over-interpretation of data will overestimate real risk for the patient. Just because a chemical has the capacity, or even potential, to interfere with mitochondrial function, does not mean that adverse outcomes will invariably occur. The degree of exposure is critical to real risk. The field of toxicology typically utilized high exposures to evaluate risk, using exposure levels that exceed typical exposures (slide 27).

The threshold for concern when using a medication known to be potentially toxic to the mitochondria yet has some benefit for the patient warrants a discussion with the medical physician or team. A discussion about the level of risk vs. benefit fro the individual needs to be specifically addressed. The level of risk begins with laboratory studies, looking at dose related responses in a non-human model to find a dose that does not interfere with the mitochondria. Once safe dose ranges are established, the drug is studied in humans.

In summary, chemical-induced mitochondrionopathies can occur directly and indirectly at many of the phases of energy production within each cell.

Additional Reading

Drug Toxicity and Mitochondria, Dr. James Dykens.
Drug-Induced Mitochondrial Dysfunction: An Emerging Model for Idiosyncratic DrugToxicity, James A. Dykens
Mitochondrial Toxicity, Dr. Katherine Sims.

Multiple Targets for Drug-induced Mitochondrial Toxicity, Wallace K.
Dr. Bruce Cohen, "The Mitochondrial Toxicity of Prescription Pharmacopoeia."
Slide 28 also contains a detailed listed of extended readings.