Microtubule targeted chemotherapy alters mitochondrial function by changing mitochondrial permeability to ADP in skeletal muscle

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Introduction:
Mitochondria are critical for healthy muscles. These structures ensure an efficient source of energy by using glucose and fat to convert ADP to ATP (adenosine triphosphate). The ability of ADP to stimulate mitochondrial oxidative phosphorylation (respiration or oxygen consumption) and lower reactive oxygen species (H$_2$O$_2$) emission is partially dependent on its transport through VDAC on the outer mitochondrial membrane. Emerging evidence suggests microtubules - a key component to the cytoskeleton - can regulate ADP diffusion into the mitochondria by physically closing or opening VDAC. However, the extent to which this model regulates mitochondrial function in muscle remains unknown. We reasoned that manipulating microtubule architecture with microtubule stabilizing (Taxol) and destabilizing (Vinblastine) compounds would provide insight into whether microtubules regulate mitochondrial function by changing VDAC permeability to ADP. Given these compounds are also used as chemotherapies, this theoretical model may also explain why muscle weakness is commonly reported with this treatment. We hypothesize that in-vivo injections of a microtubule stabilizer will limit the ability of ADP diffusion by increasing VDAC-microtubule interactions while the destabilizer will promote the import of ADP by decreasing interactions.

Methods:
Wistar rats (male) received 2 days of injections with a microtubule stabilizer (Taxol), destabilizer (Vinblastine) or control (saline). Voluntary running, grip strength, in-vivo maximum isometric torque, and fatigue was measured prior to and following injections. The ability of ADP to stimulate oxygen consumption and lower H$_2$O$_2$ emission was measured in permeabilized fibers prepared from soleus and white gastrocnemius (WG) muscles. Lastly, the microtubule-VDAC interaction was measured in sectioned soleus muscles using confocal microscopy.

Results:
Protein-protein interactions measured by confocal microscopy suggests stabilizing microtubules (taxol) decreased microtubule-VDAC interactions (more permeable) whereas destabilizing microtubules (vinblastine) increased interactions (less permeable) in the soleus muscle (WG data in progress). To determine whether these protein interactions altered mitochondrial bioenergetic function we completed assays dependent on the diffusion of ADP into the mitochondria to synthesize ATP. In soleus, neither drug changed ADP-stimulated respiration suggesting there is no relationship between microtubule-VDAC binding and mitochondrial bioenergetics in this oxidative muscle, contrary to what is expected based on the proposed model. In WG, vinblastine lowered respiration at low (25µM) [ADP] vs saline injected rats (saline; 16.5 ± 2.0, vinblastine 14.5 ± 1.1 pmols/s/mg w.w., p=0.04). To test the ability of ADP to diffuse into the mitochondria and suppress H$_2$O$_2$ emission we stimulated H$_2$O$_2$ production with 5mM succinate followed by an ADP titration. We found no differences in the ability of ADP to suppress H$_2$O$_2$ in both muscles with either drug. Lastly, to determine whether whole body or hind-limb muscle strength was altered following injections, we completed a series of functional tests. Both drugs decreased voluntary running (saline; 2.48km, Taxol; 1.20km and vinblastine; 0.37km, p=0.02) but no differences were observed in grip strength and skeletal muscle maximal torque. Interestingly, the number of contractions to reach 50% of maximum torque during a stimulated hind-limb fatigue test was much lower
with stabilizing injections and much higher with destabilizing injections when compared to saline injected rats (# of contractions to 50% of max; saline= 61-63, taxol= 48-49, vinblastine= 80-81).

**Discussion and conclusions:**
Preliminary results in the soleus muscle with injections of a microtubule destabilizer but not the stabilizer was successful at altering the microtubule-VDAC interaction. However, this was not related to altered mitochondrial function. In contrast, WG respired significantly less with the microtubule destabilizer at physiological [ADP]. Our next experiments will determine if this is due to an increased microtubule-VDAC binding as seen in the soleus microtubule-VDAC interaction measure, which may be limiting ADP diffusion into the mitochondria. The disparate and divergent results suggest the originally proposed model of microtubule-regulation of mitochondrial function may not be applicable to skeletal muscle, particularly within the context of how energy metabolism dictates muscle endurance.