The effects of CDKN1a/p21 on Oxidative Stress and Mitochondrial Function During Long Duration Spaceflight

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Background
The spaceflight environment includes several factors, including microgravity and space radiation, which result in decreased regenerative capacities and increased degeneration in multiple mammalian tissues. CDKN1a/p21 is potent cell cycle arrest molecule that is activated in response to DNA damage. Activation of CDKN1a/p21 under such conditions enables molecular mechanisms to repair DNA and enable the cell cycle to resume. Research has also shown that CDKN1a/p21 is overexpressed during spaceflight exposure which may be a consequence of increased intracellular reactive oxygen species (ROS) and mitochondrial dysfunction. Oxidative stress is also linked to cardiovascular disease as increased ROS stimulates an increase in vascular smooth muscle cell proliferation and invokes an inflammatory response, thus further linking oxidative stress to cardiovascular disease. When cells from CDKN1a/p21 (-/-) knockout mice (KO) are introduced to an environment that increases ROS but they have no regulation of cell proliferation, the inflammatory response in heightened.

Experimental Design
In this preliminary study, we sought to characterize the response of smooth muscle cells and differentiating bone marrow stem cells to oxidative stress by exposing them to H2O2 in the presence or absence of PQP. The role of CDKN1a/p21 on cellular status was investigated through the use of wildtype (WT) and CDKN1a/p21 (-/-) knockout (KO) cells.

Study 1:
Smooth muscle cells were exposed to 100 µM H2O2 with and without PQP for a period of 2 h. Following oxidative stress, the media was changed and cells were allowed to recover for 24 h and 1 week (bone marrow stem cells) with regular feeding schedules. Analysis was conducted at 2 and 24 h post-stress.

Study 2:
Bone marrow stem cells were isolated from the long bones of WT and KO mice and allowed to culture under osteoblastic conditions for 9 days. Cells were exposed to 200 µM hydrogen peroxide with and without PQP and incubated for 2 h. Analysis was conducted at 24 h and 7 d post stress.

Results
Figure 1. Research shows that oxidative stress and DNA damage is linked to CDKN1a/p21 through p53.

Figure 2. When stimulated, vascular smooth muscle cells secrete proteins that increase the rise of ROS, increasing the stress on the cells in their environment.

Figure 3. Increased production of ROS coupled with decreased antioxidant defenses give rise to oxidative stress in multiple physiological systems, including the cardiovascular system and musculoskeletal system.

Figure 4. CDKN1a/p21 is overexpressed during spaceflight, arresting cells in cell cycle and decreasing their rate of proliferation. During spaceflight conditions, increased oxidative stress may cause activation of CDKN1a/p21 and arrest of the cell cycle to enable repair of DNA damage. However, arrest of the cell cycle consequently inhibits tissue regenerative repair mechanisms through the inhibition of proliferation and differentiation.

Hypothesis
We hypothesize that CDKN1a/p21 status has a direct effect on the reaction of both smooth muscle cells and bone marrow stem cells to oxidative stress, through effects on quiescent versus active cell states. Furthermore, we hypothesize that PQP (a nutritional countermeasure) may mitigate the effects of oxidative stress through attenuation of p21 expression.

Results indicate that smooth muscle cells derived from KO mice have significantly higher membrane potential than WT counterparts. Increased membrane potential was seen in WT mice 24 h after H2O2 treatment. This is likely a result of increased metabolic activity to reduce intracellular ROS. Mitochondrial ROS levels are also higher in KO cells compared to WT. However, WT cells exhibit a spike in H2O2 treatment 24 h post H2O2 treatment, which is not seen in KO cells. Interestingly, PQP treatment decreases this peak slightly, albeit not significantly.

Conclusion
These experiments attempted to determine the role of CDKN1a/p21 in oxidative stress-induced damage during spaceflight exposure. Our preliminary data using smooth muscle cells suggest that oxidative stress has a significant effect on WT mitochondrial activity but not on KO cells. Furthermore, this effect may be mitigated by PQP treatment. Our preliminary data using differentiating bone marrow stem cells indicate a potential role for CDKN1a/p21 in oxidative stress-induced cell cycle arrest that may be mitigated by PQP treatment.

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References: