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.

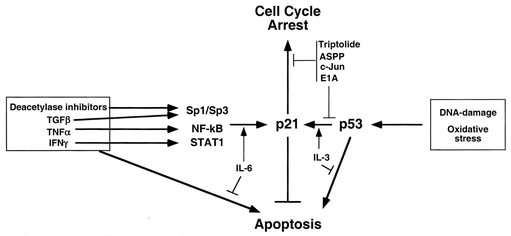


Figure 1. Research shows that oxidative stress and DNA damage is linked to CDKN1a/p21 through p53.

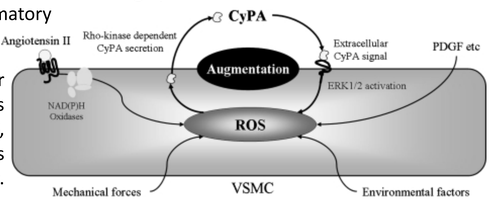
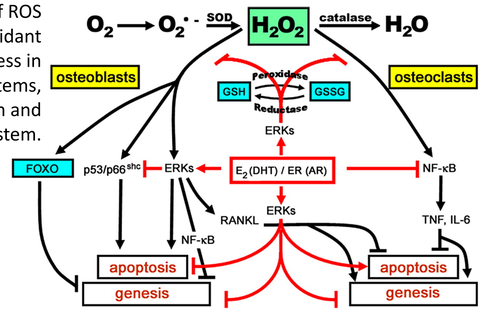


Figure 2. When stimulated, vascular smooth muscle cells secrete proteins that increase the rise of ROS, increasing the stress on the cells environment.

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



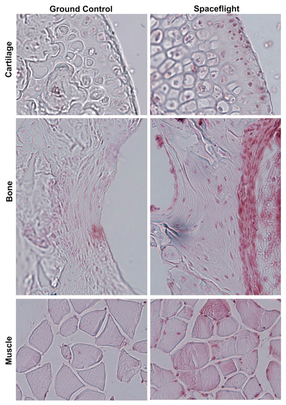


Figure 4. CDKN1a/p21 is overexpressed during spaceflight, arresting cells in cell cycle and decreasing their rate of proliferation.

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 hypothesis that PQQ (a nutritional countermeasure) may mitigate the effects of oxidative stress through attenuation of p21 expression.

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 H_2O_2 in the presence or absence of PQQ. The role of CDKN1a/p21 on cellular status was investigated through the use of wildtype (WT) and CDKN1a/p21 (-/-) knockout (KO) cells.

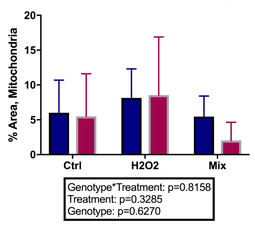
Study 1:
Smooth muscle cells were exposed to 100 μM H_2O_2 with and without PQQ for a period of 2 h. Following oxidative stress, the media was changed and cells were allowed to recover for 24 h and 1 week (bone marrow stem cells) with regular feeding schedules. Analysis was conducted at 2 h and 24 h post-stress.

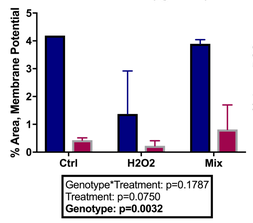
Study 2:
Bone marrow stem cells were isolated from the long bones of WT and KO mice and allowed to culture under osteoblastic conditions for 9 days. Cells were exposed to 200 μM hydrogen peroxide with and without PQQ and incubated for 2 h. Analysis was conducted at 24 h and 7 d post stress.

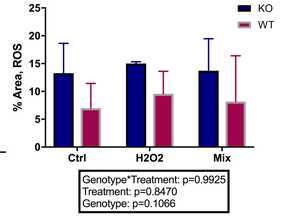
Results

Fluorescence Imaging Analysis of Smooth Muscle Cells

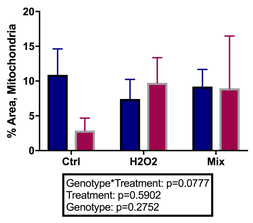
Fluorescence imaging of smooth muscle cells 2 hours and 24 hours post stress was conducted using MitoTracker mitochondrial assays. We specifically measured mitochondrial area, mitochondrial membrane potential and reactive oxygen species.

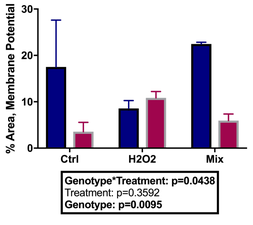


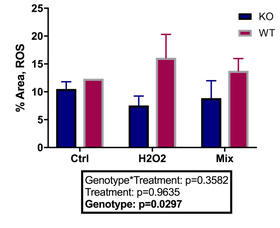




		Mitochondria			Membrane Potential			ROS	
2 hr	WT	H2O2 vs. Ctrl	637%	WT	H2O2 vs. Ctrl	-48%	WT	H2O2 vs. Ctrl	37%
		Mix vs. Ctrl	748%		Mix vs. Ctrl	88%		Mix vs. Ctrl	17%
		Mix vs. H202	15%		Mix vs. H202	260%		Mix vs. H202	-14%
	KO	H202 vs. Ctrl	-31%	KO	H202 vs. Ctrl	-67%	KO	H202 vs. Ctrl	13%
		Mix vs. Ctrl	-59%		Mix vs. Ctrl	-7%		Mix vs. Ctrl	3%
		Mix vs. H202	-41%		Mix vs. H202	184%		Mix vs. H202	-9%
	KO vs. WT	Ctrl	3394%	KO vs. WT	Ctrl	870%	KO vs. WT	Ctrl	90%
		H202	226%		H202	508%		H202	57%
		Mix	68%		Mix	379%		Mix	68%







		Mitochondria			Membrane Potential			ROS	
24 hr	WT	H2O2 vs. Ctrl	576%	WT	H2O2 vs. Ctrl	207%	WT	H2O2 vs. Ctrl	30%
		Mix vs. Ctrl	614%		Mix vs. Ctrl	69%%		Mix vs. Ctrl	11%
		Mix vs. H202	6%		Mix vs. H202	-45%		Mix vs. H202	-15%
	KO	H202 vs. Ctrl	-42%	KO	H202 vs. Ctrl	-51%	KO	H202 vs. Ctrl	-28%
		Mix vs. Ctrl	-47%		Mix vs. Ctrl	28%		Mix vs. Ctrl	-16%
		Mix vs. H202	-9%		Mix vs. H202	162%		Mix vs. H202	18%
	KO vs. WT	Ctrl	649%	KO vs. WT	Ctrl	396%	KO vs. WT	Ctrl	-15%
		H202	-36%		H202	-21%		H202	-53%
		Mix	-45%		Mix	278%		Mix	-36%

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 H_2O_2 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 H_2O_2 treatment 24 h post H_2O_2 treatment, which is not seen in KO cells. Interestingly PQQ treatment decreases this peak slightly, albeit not significantly.

Results

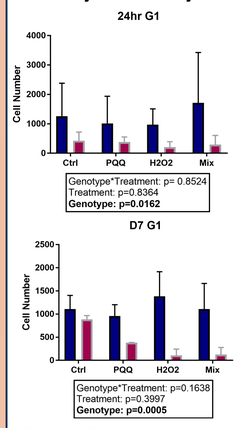
Gene Expression Analysis of Smooth Muscle Cells

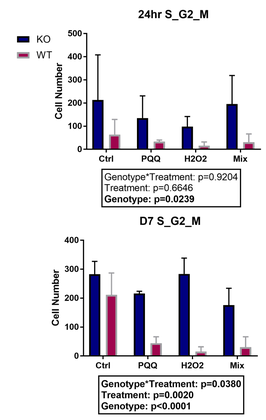
Gene expression analysis was conducted on all samples using key genes of interest relating to the cell cycle, vascular growth and oxidative stress regulators.

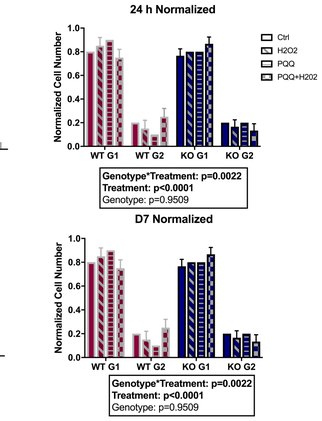
	WT ctrl vs. KO ctrl		WT H2O2 vs. KO H2O2		WT H2O2+PQQ vs. KO H2O2+PQQ		WT Ctrl vs WT H2O2		WT Ctrl vs. WT H2O2+PQQ		WT H2O2 vs. WT H2O2+PQQ		KO Ctrl vs. KO H2O2		KO Ctrl vs. KO H2O2+PQQ		KO H2O2 vs. KO H2O2+PQQ	
Gene ID	Fold Change	P-value	Fold Change	P-value	Fold Change	P-value	Fold Change	P-value	Fold Change	P-value	Fold Change	P-value	Fold Change	P-value	Fold Change	P-value	Fold Change	P-value
CDKN1a							-1.05	0.970	-1.11	0.379	-1.06	0.780						
SOD1	-1.25	0.530	-1.37	0.196	-1.26	0.477	1.07	0.956	1.33	0.583	1.24	0.524	-1.026	0.691	1.31	0.125	1.35	0.115
CCNB2	-1.56	0.438	-1.68	0.478	-1.44	0.183	-1.19	0.997	-1.09	0.753	1.10	0.861	-1.286	0.499	-1.01	0.899	1.28	0.259
CDKN2a	1.04	0.613	-1.06	0.625	-1.06	0.256	1.06	0.189	1.19	0.396	1.12	0.526	-1.032	0.806	1.26	0.070	1.30	0.100
ATF2	2.35		-3.53	0.261	-2.43	0.084	-1.31	0.735	1.05		1.19	0.913	-1.700		1.01		1.73	0.128
GDF15	-1.43	0.264	-1.97	0.149	-1.33		1.00	0.941	1.27		1.27		-1.371	0.528	1.37	0.025	1.87	0.176
CCND1	-1.97	0.452	-1.25	0.426	1.32	0.134	1.16	0.368	1.09	0.584	-1.06	0.614	-1.113	0.719	1.40	0.077	1.56	0.165
VEGFA	-1.61	0.279	-2.46	0.365	-1.83	0.150	1.14	0.683	1.25	0.445	1.09	0.902	-1.333	0.478	1.11	0.865	1.75	0.046

Gene expression shows upregulation of antioxidants in stressed WT cells while there is down regulation in vascular smooth muscle growth factor in stressed KO cells.

Cell Cycle Analysis of Bone Marrow Stem Cells







Gene Expression Analysis of Bone Marrow Stem Cells

	WT ctrl vs. KO ctrl		WT H2O2 vs. KO H2O2		WT H2O2+PQQ vs. KO H2O2+PQQ		WT Ctrl vs WT H2O2		WT Ctrl vs. WT H2O2+PQQ		WT H2O2 vs. WT H2O2+PQQ		KO Ctrl vs. KO H2O2		KO Ctrl vs. KO H2O2+PQQ		KO H2O2 vs. KO H2O2+PQQ	
Gene ID	Fold Change	P-value	Fold Change	P-value	Fold Change	P-value	Fold Change	P-value	Fold Change	P-value	Fold Change	P-value	Fold Change	P-value	Fold Change	P-value	Fold Change	P-value
CDKN1a							1.20	0.011	-1.11	0.746	-1.33	0.300	-1.20	0.229	-1.25	0.098	-1.05	0.603
SOD1	-1.82	0.043	-1.83	0.012	-1.78	0.086	1.12	0.260	1.00	0.963	-1.12	0.522	1.11	0.548	1.02	0.939	-1.09	0.288
CCNB2	-15.78												1.14	0.197	-1.05	0.545	-1.20	0.023
CDKN2a	224.41	0.423	135.77	0.063	197.40	0.008	1.45		-1.34		-1.95	0.028	-1.14	0.761	-1.53	0.262	-1.34	0.409
ATF2	1.00	0.919	2.11	0.408			-1.17	0.900					1.80	0.361	-84.16	0.255	-151.69	0.135
GDF15	4.42	0.229	3.69	0.045			-1.19	0.528					-1.43	0.514	-1.30	0.577	1.07	0.671
CCND1	1.15		-1.25				1.39						-1.04	0.815	-1.24	0.563	-1.19	0.537

	WT ctrl vs. KO ctrl		WT H2O2 vs. KO H2O2		WT H2O2+PQQ vs. KO H2O2+PQQ		WT Ctrl vs WT H2O2		WT Ctrl vs. WT H2O2+PQQ		WT H2O2 vs. WT H2O2+PQQ		KO Ctrl vs. KO H2O2		KO Ctrl vs. KO H2O2+PQQ		KO H2O2 vs. KO H2O2+PQQ	
Gene ID	Fold Change	P-value	Fold Change	P-value	Fold Change	P-value	Fold Change	P-value	Fold Change	P-value	Fold Change	P-value	Fold Change	P-value	Fold Change	P-value	Fold Change	P-value
CDKN1a							-1.16	0.432	-1.31		-1.13							
SOD1	-4.74	0.431	4.42	0.213	5.31		-9.32	0.421	-10.37		-1.11		2.25	0.302	2.43	0.402	1.08	0.793
CCNB2	39.40	0.162	3.39		34.30				3.41				1.53	0.594	2.97	0.255	1.95	0.474
CDKN2a	-2.93	0.441			-1.52		-5.42	0.407	-5.27		1.03				-1.46	0.529		
ATF2							4.69	0.420	-1.12		-5.24		1.29	0.489	1.71	0.132	1.33	0.146
GDF15	-5.20	0.119	1.12	0.640	1.10		-1.41	0.733	-1.30		1.08		4.12	0.118	4.38	0.016	1.06	0.945
CCND1	3.25	0.060	4.17	0.151	7.21		-1.43	0.318	-1.30		1.09		-1.11	0.875	1.70	0.258	1.89	0.273

Compared to WT cells, the antioxidant levels in stressed KO cells increase while stress response genes decrease.

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 PQQ 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 PQQ treatment.

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References:

1. Journal of Biological Chemistry. (2017) Skeletal Involution by Age-Associated Oxidative Stress and It's Acceleration by Loss of Sex Steroids.
2. A.L. Gartel, A.L. Tyner. (2002) The Role of Cyclin-Dependent Kinase Inhibitor p21 in Apoptosis.
3. K. Satoh, P. Nigro, BC. Berk. (2010) Oxidative Stress and Vascular Smooth Muscle Growth: A Mechanistic Linkage by Cyclophilin A.