SPACEFLIGHT EFFECTS AND MOLECULAR RESPONSES IN THE MOUSE EYE: OBSERVATIONS AFTER SHUTTLE MISSION STS-133

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ABSTRACT

Microgravity-induced cephalad fluid shift and radiation exposure are some of the stressors seen in space exploration. Ocular changes leading to visual impairment in astronauts are of occupational health relevance. Therefore, we analyzed the effects of space flight in the eyes of mice. Six mice were assigned to Flight (FLT), Animal enclosure Module (AEM), or vivarium (VIV) group, respectively. Mice were sacrificed at 1, 5 or 7 days after landing from space. One eye was used for histological and immunohistoche-mistry analysis and the other eye for gene expression profiling. 8-OHdG and caspase-3 immunoreactivity were increased in the retina in FLT samples at return(R+1) compared to AEM/VIV groups, and decreased at day 7 (R+7). β-amyloid was seen in the nerve fibers at the post-laminar region of the optic nerve in the flight samples (R+7). In addition, oxidative and cellular stress response genes were upregulated in the retina of FLT samples upon landing, and decreased by R+7. According to the results, a reversible molecular damage may occur in the retina of mice exposed to spaceflight followed by protective cellular response.

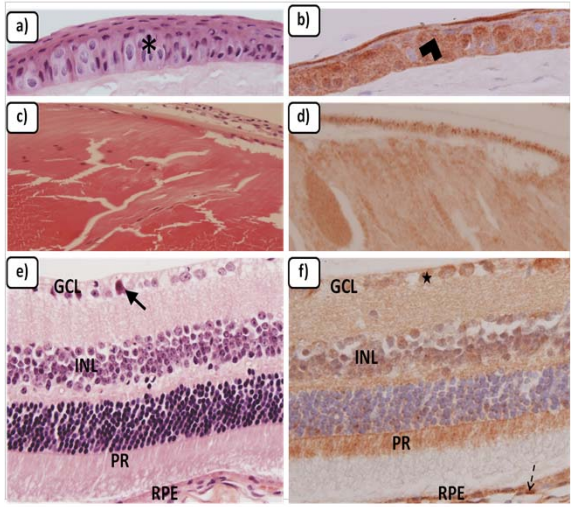
MATERIALS & METHODS

Six female albino mice were assigned to a Flight (FLT) group flown on shuttle mission STS133, Animal Enclosure Module (AEM), and vivarium (VIV) ground controls. Both FLT and AEM ground control groups were kept at AEM(self-contained habitat that provides ventilation, waste management, food, water and controlled lighting). Two mice of each group were sacrificed at 1, 5 and 7 days after the FLT group landed. One eye was fixed for histological sectioning and the contralateral eye was used for gene expression profiling by qRT-PCR. ***Histology and Immunohistochemistry-*** Sections were analyzed by hematoxylin/eosin stain and processed for 8-hydroxy-2'-deoxyguanosine (8-OHdG), caspase-3, and double staining with glial fibrillary acidic protein (GFAP) and β-amyloid. *Qualitative detection* was done by grading immunostained slides from 0 to 3+. *Quantitative detection* of 8OHdG and caspase 3 in the retina was done with NIH Image J software. ***Gene Expression Analysis-*** Mouse retina was microdissected and processed for a real-time qPCR analysis. Identified genes are in Table 1.

RESULTS

Histological Analysis. See Table 2 and Figure 1.
Immunohistochemistry. See figures 1 -3
Gene expression analysis. Gene expression of oxidative and cellular stress response genes was upregulated in the retina of FLT samples upon landing followed by lower levels by day 7(Table 1).

Figure 1



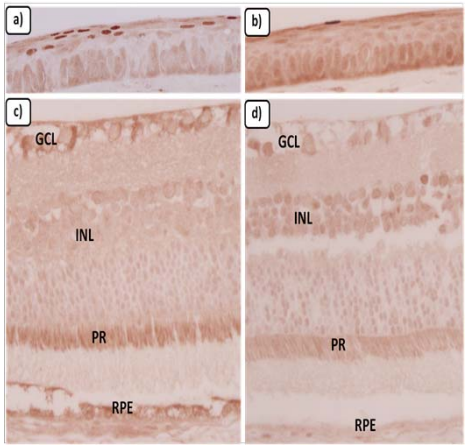
Cornea: a)H+E stain of corneal epithelium showing edema of basal layers(*) and acanthosis of all layers[AEM R+7]. b)Caspase-3 stain positivity in the superficial layers and in the basal layers (arrow head)[FLT R+1]. **Lens:** c)H+E stain showing subcapsular and cortical cataract[FLT R+1]. d)Caspase 3 positivity in lens epithelium [FLT+7]. **Retina:** e)H+E stain showing a morphologically normal retina with an apoptotic neuron(arrow)[FLT R+1]. F)Caspase 3 staining in the cytoplasm(star) in the ganglion cell layer(GCL), inner nuclear layer(INL) and photoreceptors(PR). Retinal pigment epithelium(RPE) cytoplasm is also positive (dashed arrow)[FLT R+1].

Table 1

Gene Symbol	Response	Gene name	Process
Bax	↑FLT R+1	Bcl2-associated X protein	Cell death and survival
Bcl2		B-cell lymphoma 2	
Bag1	↑FLT R+1/VIV	Bcl2-associated athanogene 1	
Atg12		Autophagy related 12	
Hsf1	↑VIV	Heat shock transcription factor 1	Cellular Stress response
Hspa1a	↓FLT R+1	Heat shock 70kDa protein 1A	
Sirt1	-	Sirtuin 1	
Nfe2l2(Nrf2)	↑VIV	Nuclear factor (erythroid-derived 2)-like 2	
Hmox1	↑FLT R+1/VIV	Heme-oxygenase 1	Oxidative stress response
Cat		Catalase	
Sod2		Superoxide dismutase 2, mitochondrial	
Gpx4	↑FLT R+1	Glutathione peroxidase 4	
Prdx1		Peroxiredoxin 1	
Cygb		Cytoglobin	
Nfkb1	↑FLT R+1/VIV	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	Inflammation
Tgfb1		Transforming growth factor beta 1	
Rpl13		Ribosomal protein L13	Normalizing genes
Rplp0		Ribosomal protein, large, P0	
Hprt		hypoxanthine phosphoribosyltransferase 1	

Genes of interest evaluated for expression changes in the mouse retina. Grouping was done according to relevant cellular processes and complete gene name with gene symbol are provided, as well as references reporting possible relevant roles in retina physiology.

Figure 2



Cornea with 80HdG stain: a)FLT R+1 mouse with with dark nuclear staining in the superficial layers and faint cytoplasmic stain in the basal cells. b)VIV R+7 mouse with dark staining in the superficial layers along with moderate cytoplasmic stain in the basal cells. **Retina with 8OHdG stain:** c)FLT R+1 mouse with staining of the cytoplasm of the ganglion cells(GCL), inner segments of photoreceptors(PR) and retinal pigment epithelium(RPE). There is faint staining of the cytoplasm of the inner nuclear layer cells(INL). d)VIV R+7 mouse with positivity in the cytoplasm GCL,, faint at the PR and negative in RPE. There is a moderate staining of the cytoplasm in the INL.

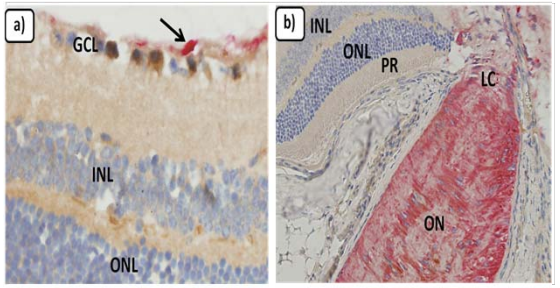
Table 2

Cornea			Lens			
FLT	AEM	VIV	FLT	AEM	VIV	
FA and E	FA	FA	Anterior subcapsular C	Nml	Anterior subcapsular C	Day 1
Bullae*, A 1+, E 2+ basal layer calcification	A* 2+	FA	Nml	Nml	Anterior subcapsular C	
FA and basal E	FA*, E 1+	Central E	Nml	Focal cortical C	Nml	Day 5
FA	Intranuclear inclusions, A 1+, E 2+	FA	Nml	Focal cortical C	Anterior subcapsular C	
FA	FA	Irregular A 1+ E 3+	Cortical C	Nml	Nml	Day 7
A* 1+, E 2+	FA	Irregular A 1+ E 2+	Cortical C	Nml	Nm	

(A)= acanthosis, (C)= cataract (E)= edema, (FA)= focal acantosis. (*)=Anterior chamber 1+ cell

Histology. Data presented per group *(FLT, AEM, VIV) and day of sacrifice(1, 5 and 7). Two mice were studied at each time point. Retina and ON were morphologically normal in both groups.

Figure 3



Double staining with Beta amyloid (brown) and glial fibrillary acid protein (GFAP) (red). a) Retina of FLT R+1 mouse. Focal cytoplasmic positivity with β-amyloid (brown) in the ganglion cell layer (GCL). Astrocytes and perivascular(arrow) positivity with GFAP(red) at GLC level. Muller cells were negative for GFAP staining. b) Retina and optic nerve of FLT R+1. GAFP was positive in the optic nerve (ON) posterior to the lamina cribrosa (LC) along with focal positivity of β-amyloid (brown)

TAKE HOME POINTS

- Exposure to space environment suggests an increased oxidative stress and cell apoptosis that decreases progressively at return. This is supported by gene expression of oxidative and cellular stress response genes.
- RPE apoptosis in mice sent to space may be the cause of retinal pathologies like retinal choroidal folds in astronauts and may increase the risk for other retinal pathologies, such as AMD.
- β-amyloid deposition at the post-laminar region of the optic nerve in mice sent to space suggests mechanical trauma, possibly associated to microgravity-induced cephalic fluid shift.
- There is the need to further investigate the nature of the changes through additional experimental work.

REFERENCES

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