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# DNA DAMAGE RESPONSE OF EX-VIVO PORCINE EYE LENSES IN ORGAN-CULTURE AND IN-VITRO CULTURED LENS EPITHELIAL CELLS TO IONIZING RADIATION

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Astronauts on space missions, especially on long-term missions to Moon or Mars have a higher risk for the expression of radiation late effects such as cancer or sub-capsular cortical eye lens opacities. This is due to higher dose and different patterns of cellular energy deposition from high-linear-energy-transfer (LET) components of galactic cosmic radiation in space than that of terrestrial low-LET radiation on Earth. The eye lens is considered to be a radiation sensitive organ with radiation induced cataract to occur with a threshold absorbed dose of 0.5 Gy of sparsely ionizing radiation. For terrestrial occupational radiation lens exposure limit is set to yearly 20 mSv by the International Commission on Radiological Protection (ICRP, Statement on tissue reactions, Ottawa, Canada, 2011). Doses perceived by astronauts are much higher: in average 150 mSv per year on the International Space Station (ISS) and 1.2 to 1.4 mSv per day on Apollo and Skylab missions (Cucinotta FA, Manuel FK, Jones J, Iszard G, Murrey J, Djojonegro B, Wear M. Space radiation and cataracts in astronauts. Radiat Res. 156:460-466, 2001).

Radiation-induced lens opacification is assumed to initiate from post irradiation proliferative activity of genetically damaged lens epithelial cells with alterations in cell cycle control, apoptosis, differentiation, and cellular disorganization, or other pathways controlling lens fiber cells' differentiation. As the porcine eye lens is similar to the human lens in size and anatomy DNA damage response was investigated in ex-vivo porcine lenses in organ culture, in in-vitro cultivated lens epithelial slabs (ES) and in porcine lens epithelial cells (pLEC). Cell survival of proliferative cells was calculated from colony forming ability (CFA) assay. The phosphorylated form of H2AX, known as $\gamma$H2AX, was used as a molecular marker to visualize DNA double strand breaks (DSB) and their repair. The modified thymidine analogue EdU was efficiently incorporated into newly synthesized DNA and visualized by a photo-stable Alexa Fluor dye in a fast, highly-specific click reaction. Propidium iodide based DNA staining for cellular DNA

content marked radiation-induced cell cycle disturbances.

Results for in-vitro cultivated pLEC are compared to in-vitro cultivated epithelial slabs and to ex-vivo porcine lenses in organ culture. The fraction of cells positive for DNA synthesis as documented microscopically for a 2 h EdU pulse was highest in pLEC, followed by ES > whole lenses. In pLEC the cell survival curve of immediate plated cells and after a recovery period of 24 h follow the equation S=1.40xD+ln 1.47 and S=1.59xD+ln 1.79, respectively. DNA DSB are induced in a dose-dependent manner ($\sim$ 18 DSB/cell/Gy) and repaired during successive recovery ($\sim$5 DSB/cell/Gy residual damage after 24 h). For doses >2 Gy a cell cycle arrest in G2 phase occurred 24 h after X-irradiation and persisted up to 72 h post-irradiation. DNA DSB induction and repair could as well be documented for ES and whole lenses after X-irradiation. In whole lenses, the amount of residual damage (after 24 h and 48 h) was highest in the equatorial zone while in the central epithelial zone DSB repair seemed to proceed with time in a manner comparable to in-vitro cultivated pLEC.

Summary: Lens organ culture allows cellular metabolism and DNA synthesis in whole lenses. Repair of DNA DSB takes place in the central epithelial layer and is reduced in the equatorial region of cultivated lenses.