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DNA DOUBLE STRAND BREAK REPAIR DURING HEAD-DOWN-TILT BED-REST: AGBRESA MEETS RADIATION

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BACKGROUND Radiation and reduced gravity impose a major burden on health and performance during human spaceflight. While radiation increases cancer risk and limits tissue regeneration, reduced gravity predisposes to musculoskeletal and cardiovascular deconditioning. Deconditioning could conceivably limit the recovery from radiation damage. Our aim was to develop a terrestrial ex vivo model that could be utilized to study the effect of simulated reduced gravity using head-down-tilt bed-rest on repair of ionizing-radiation-induced DNA damage.

METHODS We obtained blood samples from participants of the Artificial Gravity Bed Rest Study 2019 (AGBRESA) 14 days before down bed-rest (BDC -14), 30 and 57 days into bed-rest (HDT 30 and HDT 57), and after 10 days recovery (R +10). The 60 days strict head-down-tilt bedrest study compares two short-arm centrifugation protocols (interval artificial gravity = iAG; continuous artificial gravity = cAG) as a counter-measure with a control group. We assessed the repair of ex vivo induced DNA double strand breaks in human peripheral blood mononuclear cells (PBMC). PBMC isolated through Histopaque-1077 in density gradient centrifugation were exposed to 1 and 4 Gy X-rays to induce DNA double strand breaks. Cells were fixed 0.5, 1, 2, 4 and 24 h after X-irradiation. DNA double strand breaks were detected via immunofluorescence staining of γH2AX and quantified using flow cytometry.

RESULTS At BDC -14 the relative γH2AX fluorescence signal of PBMC increased with an intensity peak at 2 h post-irradiation (1 Gy: 2.79 $\pm$ 0.60, 4 Gy: 4.76 $\pm$ 1.20, n = 15). Repair of the DNA damage can be observed with a decreased γH2AX signal after 4 h (1 Gy: 2.14 $\pm$ 0.50, 4 Gy: 4.04 $\pm$ 0.91, n = 15). The signal for 1 Gy X-irradiated PBMC returned to baseline after 24 h (0.96 $\pm$ 0.24, n = 15), while exposure to 4 Gy X-rays resulted in residual damages (1.53 $\pm$ 0.49, n = 15). At HDT 30, the γH2AX signal at peak intensity (2 h post-irradiation) decreased compared to BCD -14 (1 Gy: 2.43 $\pm$ 0.78, 4 Gy: 3.77 $\pm$ 1.20, n = 12). This decrease is significant for 4 Gy (p = 0.02). Similar to BDC -14, later time points show a reduced signal with a return to baseline after 24 h for 1 Gy (0.93 $\pm$ 0.14, n = 12) and residual damage for 4 Gy (1.29 $\pm$ 0.25, n = 12). At HDT 57, a further signal decrease is observed. At peak (2 h post-irradiation), the intensity was significantly lower than at HDT 30 (1 Gy: 2.43 $\pm$ 0.78 / p = 0.005, 4 Gy: 3.77 $\pm$ 1.20 / p = 0.009, n = 12). As before, exposure to 1 Gy leads to a reduction of the γH2AX signal to baseline levels (0.92 $\pm$ 0.18, n = 12), while 4 Gy exposure results in residual damage (1.30 $\pm$ 0.34, n = 12). At R +10, the signal decrease remained at the level of HDT 57. At peak intensity 2 h after radiation exposure, no significant modulation of the signal was observed (1 Gy: 2.38 $\pm$ 0.38, 4 Gy: 3.90 $\pm$ 0.40, n = 12). The reduction to baseline after 24 h for 1 Gy exposure appears to be comparable to HDT 57 (1.02 $\pm$ 0.17, n = 12) as well as the residual damage after 4 Gy exposure (1.48 $\pm$ 0.25, n = 12). Comparison of the counter-measure groups revealed no significant difference between study time points.

CONCLUSION The results from BDC -14 provide a baseline for DNA double strand break repair capacity of human PBMC. The steadily decreasing signal during the bed rest phase indicates an effect of the study conditions on DNA damage repair. A recovery time of 10 days appears to be insufficient to alleviate this effect. In order to avoid study-based confounding factors, additional endpoints, more time points, internal controls and control groups are recommended for further studies. The ex vivo model that was successfully established in this study will be implemented in further human bed rest and intervention studies.

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