A ground-based evaluation of the impact of neutron dose rate on health effects during space travel

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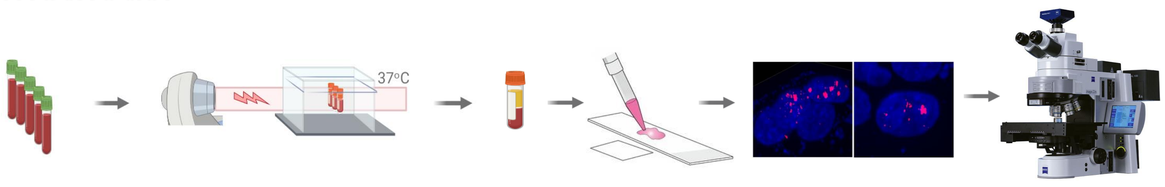
Introduction: The lack of information on how biological systems respond to low-dose and low dose-rate radiation makes it difficult to accurately assess the corresponding health risks. This is of critical importance to space radiation, which remains a serious concern for long-term manned space exploration. A growing number of particle accelerator facilities implement ground-based analogues to study the biological effects of simulated space radiation. Here, we introduce first results of a project on the "Optimization and validation of a unique ground-based in vitro model to study space health effects" (INVEST) at iThemba LABS, which aims to implement a first ground-based set-up to study space health effects in Africa. The focus of this work is on neutron irradiation, which is considered to be an important secondary component in space radiation fields. In both studies, irradiations were conducted with p(66)/Be(40) neutron irradiation (fluence-weighted average energy: 29.8 MeV) at a lower dose rate (LDR) of 0.015 Gy/min or a higher dose rate (HDR) of 0.400 Gy/min.

Study 1: The impact of Neutron dose rate on DNA repair kinetics

The γ-H2AX foci assay is an immunohistochemistry technique used to monitor DNA double-strand break (DSB) induction. Here, the effect of neutron dose rate on DNA repair was investigated over a 24hr period.

Methods:



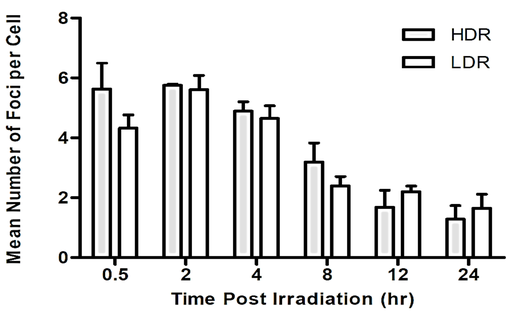
Blood samples are collected and irradiated with a range of neutron doses (0,125; 0,25; 0,5; 1 and 2Gy) at a higher and lower dose rate, in a 37°C water phantom. Lymphocytes are isolated from the blood culture by centrifugation on a density gradient medium before being fixed to a microscope slide for antibody-mediated γ-H2AX foci staining. After counterstaining with DAPI, the slides are loaded onto an automated fluorescent microscope for to count the number of foci per 1000 cells, for each dose point, per dose rate. The decay in the number of foci per cell over the period of 24hrs gives an overview of the DNA repair kinetics in response to the various radiation doses and administered dose rates and enables us to better understand the complexity of the induced DSBs.

Key findings:

γ-H2AX foci formation was 40% higher at HDR exposure samples compared to LDR exposure.

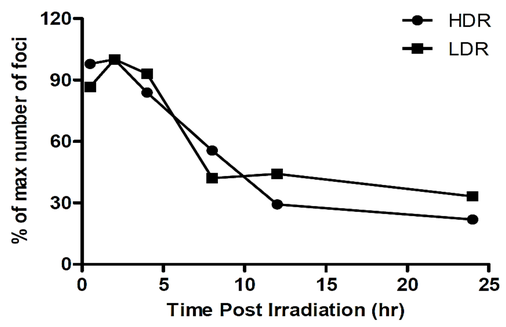
Ratio of the mean number of foci measured at different dose points (HDR/LDR).

Dose (Gy)				
0.125	0.250	0.500	1.000	2.000
1.22	1.87	1.44	1.30	1.16



The maximum γ-H2AX foci levels decreased gradually to 1.65±0.64 foci/cell (LDR) and 1.29±0.45 (HDR) at 24hr post irradiation, remaining significantly higher than background levels.

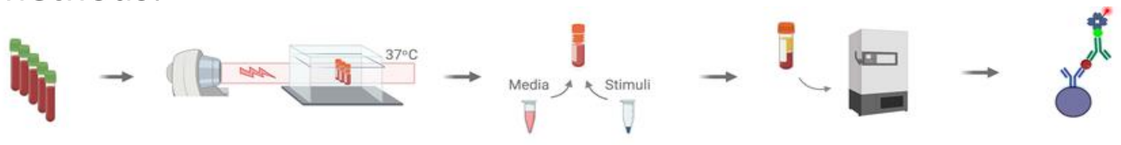
The DSB repair half-life of LDR exposure was slower than that of HDR exposure.



Study 2: The impact of Neutron dose and dose rate on immune system cytokine production

The cytokine secretion assay monitors the plasma titres of specific immune system signal markers in response to (in this study) various neutron doses and rates. Here, recall antigens and mitogens were used to stimulate immune cells post-irradiation and the effects of neutrons on the production of pro-inflammatory (Tumor Necrosis Factor-α (TNF-α), Interferon-γ (IFN-γ) and Interleukin-2 (IL-2)) and anti-inflammatory (Interleukin-10 (IL-10)) cytokines were observed.

Methods:



Blood samples were collected and irradiated as previously described before cell culture media and immune cell stimuli were added to activate the immune system, before these whole blood cultures were incubated at 37°C with 5% CO_2 and 95% humidity. After 24hrs, blood plasma was harvested by centrifugation and storage at -80°C. Later, a Luminex assay was to measure plasma-cytokine titres in comparison to the sham irradiated control baseline measurements. Repeated measures one-way ANOVA statistical tests were conducted and graphs were plotted to indicated significant relationships (P <0,05). Pooled data analysis indicated the overall effect of the higher and lower dose rate on cytokine secretion from stimulated, irradiated immune cells.

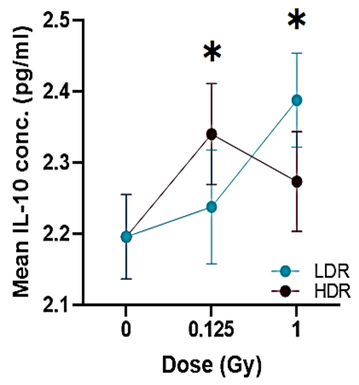
Key findings:

In a pooled analysis, the HDR significantly increased IL-2 titres (under PWM-stimulation) and IFN-γ titres (with all stimulants), but significantly decreased TNF-a secretion in unstimulated cultures.

Rate comparison

Cytokine	Stimulant	Agent	Effect on titre vs. LDR	*P*-Value
IFN-γ	PWM, HKLM or LPS	HDR	Decrease	<0.01
IL-2	PWM	HDR	Decrease	<0.05
TNF-α	None	HDR	Increase	<0.01

After PWM-stimulation, IL-10 levels were significantly increased at 0.125Gy HDR and 1Gy LDR.



Conclusion: The upcoming Mars mission will expose astronauts to a significant proportion of neutrons, generated by spallation, at a constant low rate. Together these studies indicate the potential of fast neutrons to produce sustained DNA damage, which may accumulate and result in oncogenesis. The repair kinetics analysis highlights longer damage decay rate for LDR neutron exposures, suggesting that the dose rate may be inversely related to DNA DSB complexity. The significant flip in IL-10 plasma titres in response to varied neutron doses or dose rates, indicates a complex sensitivity of the anti-inflammatory cell-medicated immune response to these factors and their ability to dysregulate the immune system. A clear dose rate effect is seen in the pooled data analysis of IFN-γ and IL-2 secretion from the stimulated immune system while the significant change in TNF-α concentrations is a direct response of the unstimulated immune system to the change in neutron dose rate. Altogether, these findings suggest that dose rate is a crucial component for cancer risk calculations for deep space missions.

References

Study 1: Nair S, Engelbrecht M, Miles X, et al. The Impact of Dose Rate on DNA Double-Strand Break Formation and Repair in Human Lymphocytes Exposed to Fast Neutron Irradiation. Int J Mol Sci. 2019;20(21):5350. Published 2019 Oct 28. doi:10.3390/ijms20215350

Study 2: Fisher, Randall, et al. Immunological changes during space travel: a ground-based evaluation of the impact of neutron dose rate on plasma cytokine levels in human whole blood cultures. Frontiers in Physics 8 (2020): 396.