

# Comparison of chromosome aberration frequencies in pre- and post-flight astronaut lymphocytes irradiated *in vitro* with gamma rays

H. Wu<sup>1,2</sup>, K. George<sup>1,3</sup>, V. Willingham<sup>1,3</sup>, F.A. Cucinotta<sup>1</sup>

1. Radiation Biophysics Laboratory, NASA Johnson Space Center, Houston, TX 77058 (USA)

2. Kelsey-Seybold Clinic, NASA Johnson Space Center, SD23, Houston, TX 77058 (USA)

3. Wyle Laboratories, 1290 Hercules Drive, Houston, TX 77058 (USA)

## Abstract

If radiosensitivity is altered in a microgravity environment, it will affect the accuracy of assessing astronauts' risk from exposure to space radiation. To investigate the effects of space flight on radiosensitivity, we exposed a crewmember's blood to gamma rays at doses ranging from 0 to 3 Gy and analyzed chromosome aberrations in mitotic lymphocytes. The blood samples were collected 10 days prior to an 8-day Shuttle mission, the day the flight returned, and 14 days after the flight. After exposure, lymphocytes were stimulated to grow in media containing phytohaemagglutinin (PHA) and mitotic cells were harvested for chromosome analysis using a fluorescence *in situ* hybridization (FISH) with whole chromosome specific probes. The dose response of total exchanges showed no changes in the radiosensitivity after the mission.

KEYWORDS: Radiation, space flight, chromosoma aberration.

## 1. Introduction

Microgravity in space has been shown to alter gene expression [1] and can potentially influence the repair of DNA damaged induced by radiation. A synergistic effect of microgravity and space radiation would have a major impact on the risk assessment for space travel. Although it has been shown that radiosensitivity is enhanced during spaceflight in some cases (see ref. [2] for a review), such a synergistic effect was challenged by the results of other studies. In an experiment where cells were irradiated then frozen prior to a mission and incubated in space, Horneck et al. [3] did not observe significant differences in the kinetics of DNA breakage rejoining and cell survival between the microgravity samples and the corresponding controls. Similar results were also obtained by Pross et al. after exposing yeast cells in space with a beta-particle emitter [4].

In the present study, we investigated the synergistic effect of microgravity and radiation on the induction of chromosome aberrations in crewmembers' peripheral blood lymphocytes. Microgravity has been shown to influence the growth kinetics of lymphocytes [5]. In a previous study of astronauts after 3-5 month Mir missions, Yang et al. reported that the lymphocytes collected immediately after mission grew slower in growth medium than the samples collected before the mission [6]. It took about 2 weeks for the growth rate to recover to the pre-flight level. This observation suggested that if the radiosensitivity was altered in space, it may take a certain amount of time to recover to the preflight level and, thus, we would be able to observe changes in radiosensitivity by exposing astronauts' blood

samples *in vitro* to ground-base radiation immediately after mission.

Synergistic effects of microgravity and spaceflight factors on the induction of chromosome aberrations in human lymphocytes have been investigated in the early days of the manned space program. During Gemini III and Gemini XI missions, Bender et al. exposed human lymphocytes to a beta source in space and compared the chromosome aberrations induced during flight with ground samples [7, 8]. Although the Gemini III study showed a higher yield of single-break aberrations in the flight samples, this result was not confirmed in the Gemini XI experiment. In the Gemini experiments, the blood was drawn from test subjects several days prior to launch and irradiated several hours after launch. In the present study, however, blood samples were collected from a crewmember immediately after return of a Shuttle mission and were exposed *in vitro* to a gamma source.

## 2. Materials and methods

STS-103 was a 8-day Shuttle mission at 28.5 degree inclination and 586 km altitude. This Hubble Space Telescope servicing mission had one of the highest altitudes of the Shuttle missions and an average crew TLD dose of 1.6 cGy. Blood from a crewmember of STS-103 was drawn into a vacutainer tube containing Sodium Heparin (100 U.S.P. units) at three different times: 10 days prior to the launch (L-10), the day the flight returned (R+0) and 14 days after landing (R+14). For the L-10 and R+14 samples, whole blood was kept on ice and irradiated with <sup>137</sup>Cs  $\gamma$  rays at a dose rate of 10 Gy/min. After

irradiation lymphocytes were cultured in RPMI medium supplemented with 20% calf serum and 1% phytohemagglutinin (PHA), and were incubated at 37°C for 48 h. Chromosomes were collected by adding colcemid (0.2 µg/ml) to the cultures and the cells were incubated for an additional 2 hours. Metaphase Samples were then swollen in 0.075 M KCl solution at 37°C for 20 minutes, fixed in methanol/acetic acid (3:1 vol/vol) fixative solution and stored at -20°C. The R+0 samples were collected 2 hours after landing, kept on ice and brought from Kennedy Space Center to Johnson Space Center. The samples were irradiated with the same γ source 22 hours after landing and incubated at 37°C for 60 hours in growth medium before colcemid was added for collection of mitotic cells. The longer incubation time was designed to counter the slower growth rate of lymphocyte cells experienced after a long duration mission.

Chromosome aberrations were analyzed using the FISH technique with chromosome #1 and #5 probes. Hybridized spreads were counterstained in DAPI and viewed with a Zeiss Axioplan fluorescence microscope.

### 3. Results

Results of the study are shown in Table I. The preflight background frequency for total exchanges was  $3/2962 = 0.001$  and the post flight was background frequency  $10/4287 = 0.002$ . The background frequencies were negligible compared to the frequencies of chromosome aberrations induced by 1-3 Gy of γ rays. The comparison of dose response curves for total exchanges pre- and post-flight is shown in Figure 1. Total exchanges included complete, incomplete and complex exchanges. It has

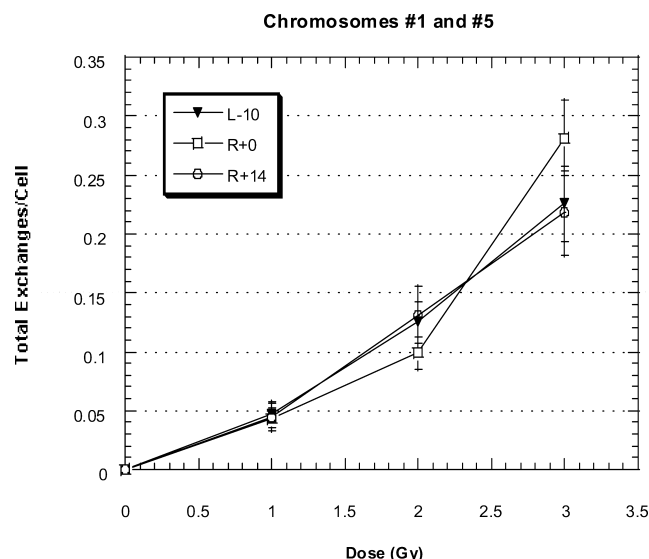


Fig. 1 – Dose response of total exchanges involving chromosomes #1 and #5 in crewmember’s lymphocytes irradiated *in vitro* with γ rays pre- and post-flight. Total exchanges included complete, incomplete and complex exchanges.

been shown that most of the incomplete exchanges are in fact falsely scored because some exchanges are too small to be detected under the microscope [9]. As a result, the frequency of incomplete exchanges may vary depending on the degree of chromosome condensation and sometimes slide preparation. Therefore, total exchanges are a better indicator when comparison of chromosome aberration frequencies is made. No significant differences of total exchanges was found between pre- and post-flight samples, as indicated in Figure 1.

**Table I** – Aberrations in chromosomes #1 and #5 of crewmember’s lymphocyte cells irradiated *in vitro* with γ rays pre- and post-flight.

Sample	Dose (Gy)	Cells scored	Complete exchange	Incomplete exchange	Complex exchange	Centric ring	Excess Acentric fragment
L-10	0	2962	1	0	2	0	0
L-10	1	402	10	8	1	0	0
L-10	2	369	27	17	2	0	8
L-10	3	217	30	12	7	1	9
R+0	0	4287	7	2	1	0	1
R+0	1	466	18	2	0	1	1
R+0	2	476	28	17	2	5	11
R+0	3	281	32	24	13	5	12
R+14	1	317	7	7	0	0	1
R+14	2	222	23	4	2	1	3
R+14	3	170	24	9	4	3	12

#### 4. Discussion

There was no change in radiosensitivity of crewmembers' lymphocytes, measured using chromosome damage induced by in vitro  $\gamma$  ray exposures received within 24 hours of return from an 8-day mission. This result was in agreement with the findings reported by Bender et al. [8], Honeck et al. [3] and Pross et al [4], and indicated that the response of cells to radiation after a short-duration mission is similar to the response on the ground.

Whether DNA repair damage capability is altered after long-duration missions is still an open question. Certain changes in the performance of cells were indeed found to be dependant on the length of the mission. For instance, Konstantinova et al. reported that the decrease in the PHA reactivity of T cells in cosmonauts after prolonged space flights was more significant than after 7-10 day missions [10]. Analysis of chromosome aberrations in crewmembers' lymphocyte cells after 3-5 month Mir missions showed a RBE of 3.5 while the Q value calculated from the tissue equivalent proportional counter (TEPC) was lower [6]. One possible explanation for the difference in the RBE is that the TEPC measurement lacked the contribution from the secondary neutrons. A possible enhancement in the radiosensitivity after long-duration missions could provide an alternative explanation.

The average crew TLD dose for the STS-103 mission was 1.6 cGy. It has been shown previously that human lymphocytes exposed to low doses of X-rays are less susceptible to the induction of chromatid breaks by high doses of X rays [11]. This adaptive response was reported when the initial exposure was as low as 1 cGy. However, in the present study, no adaptive response was found when the initial dose of 1.6 cGy was received over a period of 8 days in flight. Unlike most of the studies

of the adaptive response, the 1.6 cGy of space radiation in the present case was composed of protons and a small fraction of heavy ions, and the challenging dose was given about 22 hours after the Shuttle landed.

#### REFERENCE

- [1] Hammond TG, Lewis FC, Goodwin TJ, Linnehan RM, Wolf DA, Hire KP, Campbell WC, Benes E, O'Reilly KC, Blobus RK, Kaysen JH. Gene expression in space. *Nature Medicine*, 1999; 5; 359.
- [2] Horneck G. Radiobiological experiments in space: a review. *Nucl Tracks Radiat Meas* 1992; 20; 185-205.
- [3] Horneck G, Rettberg P, Kozubek S, Baumstark-Khan C, Rink H, Schaefer M, Schmitz C. The influence of microgravity on repair of radiation-induced DNA damage in bacteria and human fibroblasts. *Radiat Res* 1997; 147; 376-384.
- [4] Pross HD, Casares A, Kiefer J. Induction and repair of DNA double-strand breaks under irradiation and microgravity. *Radiat Res* 2000; 153; 521-525.
- [5] Cogolli A, Tschopp A. Effect of spaceflight on lymphocyte stimulation. *The Physiologist* 1980; 23; S63-66.
- [6] Yang TC, George K, Johnson AS, Durante M, Fedorenko BS. Biodosimetry results from space flight Mir-18. *Radiat Res* 1997; 145; S17-23.
- [7] Bender MA, Gooch PC, Kondo S. The Gemini-3 S-4 spaceflight-radiation interaction experiment. *Radiat Res* 1967; 31; 81-111.
- [8] Bender MA, Gooch PC, Kondo S. The Gemini XI S-4 spaceflight-radiation interaction experiment: The human blood experiment. *Radiat Res* 1968; 34; 28-238.
- [9] Wu H, George K, Yang TC. Estimate of the frequency of true incomplete exchanges in human lymphocytes exposed to 1 GeV/u Fe ions in vitro. *Int J Radiat Biol* 1999; 75; 593-599.
- [10] Konstantinova IV, Rykova MP, Lesnyak AT, Antropova EA. Immune changes during long-duration missions. *J Leukocyte Biology* 1993; 54; 189-201.
- [11] Wolff S, Afzal V, Wiencke JK, Olovieri G, Michaeli A. Human lymphocytes exposed to low doses of ionizing radiations become refractory to high doses of radiation, as well as chemical mutagens that induce double-strand breaks in DNA. *Int J Radiat Biol* 1988; 53; 39-48.