J. RADIAT. RES., 43: SUPPL., S129–S132 (2002)

Analysis of Complex-type Chromosome Exchanges in Astronauts' Lymphocytes after Space Flight as a Biomarker of High-LET Exposure

KERRY GEORGE[1,2]*, HONGLU WU[1,2], VERONICA WILLINGHAM[1,2] and FRANCIS A. CUCINOTTA[2]

FISH / Chromosomes / High-LET / PCC

High-LET radiation is more efficient in producing complex-type chromosome exchanges than sparsely ionizing radiation, and this can potentially be used as a biomarker of radiation quality. To investigate if complex chromosome exchanges are induced by the high-LET component of space radiation exposure, damage was assessed in astronauts' blood lymphocytes before and after long duration missions of 3–4 months. The frequency of simple translocations increased significantly for most of the crewmembers studied. However, there were few complex exchanges detected and only one crewmember had a significant increase after flight. It has been suggested that the yield of complex chromosome damage could be underestimated when analyzing metaphase cells collected at one time point after irradiation, and analysis of chemically-induced PCC may be more accurate since problems with complicated cell-cycle delays are avoided. However, in this case the yields of chromosome damage were similar for metaphase and PCC analysis of astronauts' lymphocytes. It appears that the use of complex-type exchanges as biomarker of radiation quality *in vivo* after low-dose chronic exposure in mixed radiation fields is hampered by statistical uncertainties.

INTRODUCTION

High-LET radiation induces a higher yield of complex-type chromosome exchanges than equivalent doses of low-LET radiation[1,2]. A single hit to a cell from a densely ionizing radiation track is thought be sufficient to produce complex chromosome damage[3], whereas gamma rays do not produce a significant increase in the yield of complex rearrangements at doses below 2 Gy[4]. It follows therefore that an increase in complex chromosome damage can potentially be used as a biomarker of exposure to low doses of high-LET radiation such as those encountered by astronauts during space flight. However, difficulties may arise in accurately estimating radiation-induced complex chromosome damage at first mitosis post irradiation. Previous studies have indicated that there is a delay in the expression of chromosome damage in metaphase that is mostly related to the late emergence of complex-type damage[5–7]. It was suggested that the yield of complex chromosome damage could be underestimated when analyzing metaphase cells collected at one time point after irradiation, and chemically-induced premature chromosome condensation (PCC) may be a more accurate system since problems with complicated cell-cycle delays are avoided[5].

Although heavy particles contribute only a few percent of an astronauts' overall radiation dose during space flight[8, 9], the effect on biological damage and health risk may be significant. An increase in the yield of complex exchanges after flight could estimate the level of exposure to heavy particles. A positive link has been established between chromosome damage and cancer incidents[10], and increased frequencies of damage can be used as an estimate of the health risks associated with exposure to radiation[11,12].

Here we have obtained direct *in vivo* measurements of complex chromosome damage in blood lymphocytes of astronauts before and after long duration missions on Mir or the International Space Station (ISS), and have compared the frequencies measured in PCC and metaphase.

*Corresponding author: Phone: +1–281–483–9593
Fax: +1–281–483–3058
E-mail: kerry.a.george1@jsc.nasa.gov
[1] Wyle Life Sciences, Houston, TX, USA;
[2] NASA Johnson Space Center, Houston, TX, USA.

MATERIALS AND METHODS

Crewmembers' blood samples were collected in vacutainer tubes containing sodium heparin. Whole blood cultures were initiated in RPMI 1640 medium (Gibco BRL, Rockville, MD, USA) supplemented with 20% calf serum and 1% phytohemagglutinin (Gibco BRL, Rockville, MD, USA), and were incubated at 37°C for 48 hours. After a 2-hour treatment with 0.2 μg/ml colcemid, mitotic spreads were collected using standard cytogenetic procedures, and fixed in methanol/acetic acid (3:1, vol:vol). PCC were collected after a 30-minute treatment with 50 nM of Calyculin-A (Wako Chemicals, Japan) using the method described by Durante *et al.*[13].

A 0.5-ml volume of blood from pre- and post-flight samples was cultured with 10 μm of bromodeoxyuridine (BrdU) and a differential replication staining procedure was completed on chromosomes from these samples by incubating slides in 0.5mg/ml of Hoescht during exposure to black light (General Electric 15T8/BL bulb). Spreads were stained with Giemsa to visualize replication rounds. The percentage of cells in first mitosis was greater than 80% for all samples analyzed.

Chromosomes were hybridized *in situ* with either two or three fluorescence-labeled chromosome-specific DNA probes in different colors; spectrum green, spectrum orange, and yellow, (i.e., a 1:1 combination of green and orange probes that fluoresces yellow under a triple-band-pass filter set). All probes were obtained from Vysis (Woodcreek, IL, USA) and manufacturer's recommended procedures were used. Chromosomes were counterstained in 4′,6-diamidino-2-phenylindole (DAPI) and analyzed using a Zeiss Axioskop fluorescence microscope.

All slides analyzed in this study were coded and scored blind. Complex exchanges were scored when it was determined that an exchange involved a minimum of three breaks in two or more chromosomes. Total exchanges were calculated by adding the number of apparently simple translocations, dicentrics, incomplete translocations, incomplete dicentrics, and complex exchanges. For direct comparison of data, yields of exchanges in individual chromosome combinations were extrapolated to whole genome equivalents using the formula of Lucas *et al*[14].

RESULTS

Table 1 shows the frequency of chromosome aberrations

Table 1. Chromosome aberrations in crewmembers' lymphocytes before and after long duration Mir flights, measured in metaphase cells. Values for WGE represent whole genome equivalent values for exchanges. S.E. represents values for standard error. Only crewmember 1 has a significant increase in complex exchanges postflight.

Crew member	Sample collection	Cells scored	Chromosomes analyzed	Apparently simple translocations		Complex exchanges	
				No.	WGE ($X10^3$±S.E.)	No.	WGE ($X10^3$±S.E.)
1	Before flight	4404	1 + 2	19	15.0±3.5	1	0.7±0.7
	10 days after flight	6556	1 +2	27	14.9±2.8	7	3.8±1.4
2	Before flight	1892	1, 2 + 4	5	6.3±2.8	1	1.2±1.2
	12 days after flight	4677	2 +1	20	15.0±3.5	2	1.4±1.2
3	Before flight	3995	2 + 4	4	3.8±1.9	0	0.0±0.0
	Day of return	4056	2 + 4	9	8.4±2.7	2	1.9±1.1
4	Before flight	3792	2 + 4	12	12.2±3.4	3	3.0±1.9
	9 days after flight	4843	2 + 4	30	23.6±4.2	3	2.3±1.5
5	Before flight	742	2 + 4	3	15.2±8.7	2	10.3±7.2
	9 days after flight	2630	2 + 4	19	27.4±6.5	0	0.0±0.0
6	Before flight	2852	2 + 4	7	9.1±3.4	1	1.5±1.5
	Day of return	4672	2 + 4	26	21.3±4.2	1	0.8±0.8
	9 days after flight	3147	2 + 4	13	15.6±4.2	1	1.1±1.1

Table 2. Chromosome aberrations in crewmembers' lymphocytes before and after long duration flights on the international space station, measured in metaphase and PCC. In all cases chromosomes 1, 2 and 5 were analyzed. Values for WGE represent whole genome equivalent frequencies of exchanges. S.E. represents values for standard error.

Crew member	Sample collection	Sample type	Cells scored	Apparently simple exchanges		Complex exchanges	
				No.	WGE ($X10^3$±S.E.)	No.	WGE ($X10^3$±S.E.)
7	Before flight	PCC	7331	4	1.4±0.8	2	0.7±0.5
	10 days after flight	PCC	6304	13	5.4±1.5	0	0.0±0.0
		Metaphase	5233	16	7.9±2.0	2	1.0±0.7
8	Before flight	PCC	3693	2	1.4±0.4	0	0.0±0.0
		Metaphase	1741	0	0.0±	0	0.0±0.0
	15 days after flight	PCC	4023	11	7.1±0.8	2	1.3±0.9
		Metaphase	1524	6	10.2±4.2	1	1.7±1.7
9	Before flight	PCC	4604	15	8.5±2.2	4	2.2±1.1
		Metaphase	1635	6	9.5±3.9	0	0.0±0.0
	15 days after flight	PCC	996	6	15.7±6.4	2	5.2±3.8
		Metaphase	1625	10	16.0±1.9	1	1.6±1.6
10	Before flight	PCC	3049	11	9.4±2.8	2	1.7±1.2
		Metaphase	2083	8	10.0±3.5	2	0.0±0.0
	10 days after flight	PCC	3149	13	10.7±3.0	3	2.5±1.4
		Metaphase	3211	11	8.9±2.7	3	0.8±0.8

in the blood lymphocytes of astronauts before and after their respective missions of 3–4 months onboard the Mir space station. All cells were analyzed in first mitosis after stimulation in culture. Although the frequency of apparently simple reciprocal exchanges increased post flight for crewmembers 2–6, crewmember 1 was the only subject to show any significant increase in complex exchanges post flight. The total number of complex exchanges detected was very low; a total of 8 complex exchanges were detected preflight in the 20,910 cells analyzed from all crewmembers combined. After flight, 20 complex exchanges were detected in a total of 30,078 cells from all astronauts.

Table 2 shows a comparison of PCC and metaphase analysis of astronauts' blood lymphocytes before and after their respective missions of approximately 3 months onboard the ISS. Yields of translocations and complex-type exchanges were similar in PCC and metaphase samples, and there is no real difference in the frequency of complex exchanges after flight for any of the crewmembers. As with the study of Mir astronauts, there were few complex aberrations detected. Pooled data for metaphase and PCC analysis for all four ISS crewmembers revealed 6 complex exchanges preflight in a total of 24,136 cells analyzed, and 12 complex exchanges were detected in 26,065 cells collected after flight.

CONCLUSIONS

Since low doses of low-LET radiation do not induce complex exchanges, any increase in complex-type chromosome damage detected after exposure to the relatively low dose of radiation received during space missions would be due entirely to the high-LET component of the exposure. Biological measurements from induction of simple reciprocal translocations and dicentrics are consistent with predicted equivalent doses ([11,12,15–18]. However, there were few complex exchanges induced during the 3–4 month missions in space, and most individuals show no significant increase after flight. Since only two or three chromosome pairs were analyzed in this study this presents limitations associated with determining the true complexity of exchanges, and it is possible that exchanges scored as apparently simple-type actually contain hidden complex rearrangements. However

the frequency of true complex exchanges detected post flight would still be very low, and it appears that the use of complex-type exchanges as biomarker of radiation quality *in vivo* after low-dose chronic exposure in mixed radiation fields is hampered by statistical uncertainties.

REFERENCES

1. M. Durante, Y. Furusawa, K. George, G. Gialanella, O. Greco, G. Grossi G, N. Matsufuji, M. Pugliese, and T.C. Yang (1998) Rejoining and misrejoining of radiation-induced chromatin breaks. IV. Charged particles. Radiat. Res. **149**: 446–54.
2. I. Testard, B. Dutrillaux, and L. Sabatier (1997) Chromosomal aberrations induced in human lymphocytes by high-LET irradiation. Int. J. of Radiat. Biol. **72**: 423–433.
3. C. S. Griffin, S. J. Marsden, D. L. Stevens, P. Simpson, and J. R. K. Savage (1995) Frequencies of complex chromosome exchange aberrations induced by ^{238}Pu alpha-particles and detected by fluorescence in situ hybridization using single chromosome-specific probes. Int. J. of Radiat. Biol. **67**: 431–439.
4. P.J. Simpson and J.R.K. Savage (1996) Dose-response curves for simple and complex chromosome aberrations induced by X-rays and detected using fluorescence in situ hybridization. Int. J. Radiat. Biol. **69**: 429–436.
5. R. M. Anderson, S. J. Marsden, E. G. Wright, M. A. Kadhim, D. T. Goodhead, and C. S. Griffin (2000) Complex chromosome aberrations in peripheral blood lymphocytes as a potential biomarker of exposure to high-LET alpha-particles. Int. J. of Radiat. Biol. **76**: 31–40.
6. K. George, H. Wu, V. Willingham, Y. Furusawa, T. Kawata, and F.A. Cucinotta (2001) High- and low-LET induced chromosome damage in human lymphocytes; a time course of aberrations in metaphase and interphase. Int. J. Radiat. Biol. **77**: 175–183.
7. M. Scholz, S. Ritter, and G. Kraft (1998) Analysis of chromosome damage based on the time course of aberrations. Int. J. Radiat. Biol. **74**: 325–31.
8. G. D. Badhwar (1997) The radiation environment in low-earth orbit. Radiat. Res. **148**: S3–10.
9. H. Nikjoo, I. G. Khvostunov, and F. A. Cucinotta (2002) The Response of Tissue-Equivalent Proportional Counter to Heavy Ions. Radiat. Res. **157**: 435–445.
10. L. Hagmar, S. Bonassi, U. Stömberg, A. Brøgger, L. Knudse, H. Norppa, and C. Reuterwall (1998) Chromosomal aberrations in lymphocytes predict human cancer – a report from the European Study Group on Cytogenetics Biomarkers and Health. Cancer Res. **58**: 4117–4121.
11. F. A. Cucinotta, J. W. Wilson, J. R. Wilson, and J. F. Dicello (2000) Analysis of Mir-18 results for physical and biological dosimetry: shielding effectiveness in LEO. Radiat. Meas. **32**: 181–191.
12. M. Durante, S. Bonassi, K. George, and F. A. Cucinotta (2001) Risk Estimation Based on Chromosomal Aberrations Induced by Radiation. Radiat. Res. **156**: 662–667.
13. M. Durante, Y. Furusawa, H. Majima, T. Kawata, and E. Gotoh (1999) Association between G2-phase block and repair of radiation-induced chromosome fragments in human lymphocytes. Radiat Res. **6**: 670–676.
14. J. N. Lucas, M. Poggensee, and T. Straume (1992) The persistence of chromosome translocations in a radiation worker accidentally exposed to tritium. Cytogenet. Cell Genet. **60**: 255–6.
15. K. George, M. Durante, H. Wu, V. Willingham, G, Badhwar, and F. A. Cucinotta (2001) Chromosome Aberrations in the Blood Lymphocytes of Astronauts after Space Flight. Radiat. Res. **156**: 731–738.
16. G. Obe, I. Johannes, C. Johannes, K. Hallman, G. Reitz, and R. Facius (1997) Chromosomal aberrations in blood lymphocytes of astronauts after long-term space flights. Int. J. Radiat. Biol. **72**: 727–734.
17. I. Testard, M. Ricoul, F. Hoffschir, A. Flury-Herard, B. Dutrillaux, B. Federenko, V. Gerasimenko, and L. Sabatier (1996) Radiation-induced chromosome damage in astronauts' lymphocytes. Int. J. Radiat. Biol. **70**: 403–411.
18. M. S. Sasaki, I. Hayata, N. Kamada, Y. Kodama, and S. Kodama (2001) Chromosome aberration analysis in persons exposed to low-level radiation from the JCO criticality accident in Tokai-mura. J. Radiat. Res. **42**: S107–116.

Received on June 10, 2002
Revision on December 4, 2002
Accepted on December 9, 2002