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X-ray-induced chromosome aberrations in human lymphocytes *in vitro* are potentiated under simulated microgravity conditions (Clinostat)

P. Mosesso[1], M. Schuber[2], D. Seibt[2], C. Schmitz[2], M. Fiore[1], A. Schinoppi[1], S. Penna[1], F. Palitti[1]

1. *University of Tuscia, Department of Agrobiology and Agrochemistry, I-01100 Viterbo (Italy)*
2. *DLR Institute of Aerospace Medicine, MUSC, D-51170, Köln (Germany)*

Abstract

The influence of simulated microgravity weightlessness on the outcome of radiation-induced chromosomal aberrations was investigated using the clinostat as a tool to simulate weightlessness conditions. Treatments were performed in the G_0 phase of human lymphocytes with 1.5 Gy of X-rays alone or in combination with the DNA synthesis inhibitor of 1-β-D-arabinofuranosylcytosine (ara-C) to check also for possible specific radiation-induced DNA repair processes impairment (excision repair caused by base damage) under microgravity conditions.

The results obtained, which confirmed previous findings, showed significantly higher increases of aberrant cells and hence total number of aberrations compared to the parallel treatments performed 'on ground'. For what concern ara-C its contribution in terms of potentiation in the induction of aberrant cells was equivalent in absolute terms under simulated microgravity conditions and 'on ground' indicating that excision repair caused by base damage and inhibited by ara-C is not affected by simulated microgravity.

A possible explanation for this outcome could quote two major factors:
i) Enhanced probability that under simulated microgravity conditions the reactive DSB are spatially brought together to better interact, hence increasing the probability of mis-rejoining. ii) Alternatively chromatin structure could be modified under simulated microgravity conditions generating different quality and quantities of DNA lesions compared to treatments performed 'on ground'.

KEYWORDS: Simulated microgravity, chromosome aberrations, ara-C, inhibitors of DNA repair.

1. Introduction

In the coming International Space Station era it is foreseen that an increasing of humans will be challenged with long term exposure to the extraterrestrial environment in which space radiations and microgravity are relevant components. Therefore the question whether radiation effects are influenced by microgravity is an important aspect in risk estimation.

In this study, based also on our previous observation that bleomycin-induced chromosomal aberrations are enhanced under simulated microgravity conditions (clinostat) [1, 2], we aimed to evaluate the outcome of X-ray-induced chromosomal aberrations and possible specific radiation-induced DNA repair processes impairment under simulated microgravity conditions (clinostat). Accordingly, X-Ray treated cultures were challenged with the inhibitor of DNA synthesis 1-β-D-arabinofuranosylcytosine (ara-C). The rationale of this approach is that ara-C would block the refilling steps of X-ray-induced excision repair, thus producing an accumulation of unfilled single-strand gaps. These gaps will be then converted into double-strand breaks and visualized at metaphase as chromosome aberrations (CA) [3, 4]. On this base, the higher the level of X-ray-induced excision repair, the higher is the incidence of CA.

2. Materials and methods

2.1. *Cell cultures and treatment conditions*

Lymphocyte cultures were established from heparinized blood from a healthy donor in Ham's F-10 culture medium supplemented with 20% heat-inactivated foetal calf serum, 100 IU/ ml penicillin, 100 µg/ml streptomycin, 0.4 mM L-glutamine and phytohaemagglutinin 2% (HA 15, Murex, U.K.). Each treatment culture consisted of 1.5 ml growth medium and 1.5 ml whole blood.

Lymphocytes in the G_0 phase were exposed to 1.5 Gy of X-rays alone or in combination with ara-C $5\times10^{-5}M$ (Sigma Chemical Co., St. Louis, U.S.A.) in Ham's F-10 medium without phytohaemagglutinin (PHA) at 37°C, both in the clinostat and 'on ground'. The rotation lasted two hours to allow DNA repair processes to be almost completed. For 0-g simulation the cuvette clinostat at DLR MUSC was used. The usefulness of the clinostat as a simulation technique for 0-g has been reported by Schatz et al. [5]. Control (1 g) and clinostat samples were processed in sterile plastic tubes with an inner diameter of 4 mm. For the clinostat experiment the tubes were mounted on the horizontal clinostat axis and rotated at 60 rpm to achieve weightlessness conditions. In this way the remaining centrifugal acceleration a_c was reduced to $0 \le a_c \le 8 \times 10^{-3}$ g.

At the end of treatment the cultures were washed twice with pre-warmed Ham's F-10 and incubated in complete medium with PHA but supplemented with $1 \times 10^{-4} M$ deoxycytidine (Sigma Chemical Co.) to block residual effects of the remaining ara-C bound to the DNA polymerases and allow DNA replication to proceed normally. From each treated sample three cultures were set up and allowed to grow at 37°C for 48 hours in 15 ml cell culture plastic tubes. One of the cell cultures was also treated with 5-bromo-2'-deoxyuridine (BrdUrd) at 3 µg/ml to check for cell proliferation in both conditions (clinostat and ground). In the last three hours cultures received colcemid at a final concentration of 0.2 µg/ml. The harvesting of cultures and the preparation of slides were carried out following conventional cytological methods. Slides to be used for the identification of second division lymphocytes to evaluate the status of cell proliferation were stained with fluorochrome plus Giemsa technique [6]. Slides to be scored for the presence of chromosome aberrations were stained with aqueous Giemsa solution.

3. Results and discussion

The results obtained, which confirm previous findings, are collated in Table I. The incidence of chromosome-type aberrations (dicentrics, rings and fragments) are presented together with the percentage of total number of aberrations and cells bearing aberrations. The statistical significance of aberrant cells between each treatment and relevant control and between specific treatment 'on ground' versus the corresponding treatment performed in the Clinostat is also shown.

Treatment with X-rays induced significant increases of aberrant cells and total number of aberrations compared to relevant controls in both clinostat and 'on ground' treatment conditions. As expected, the frequencies of chromosome-type aberrations induced by X-rays during G_0 phase were markedly increased by post-treaments with ara-C [7-10].

However, in the cultures treated under simulated microgravity conditions (clinostat) the incidence of aberrant cells and hence total number of aberrations was significantly higher compared to the parallel treatments performed 'on ground'. For total number of aberrations, exchange-type aberrations (dicentrics and rings) were more influenced, being the ratio dicentrics-rings: fragments 'on ground' equal to 1 and approximately 0.5 under simulated microgravity conditions. Concerning ara-C it should be noted that its contribution in terms of potentiation was equivalent in absolute terms under simulated microgravity conditions and 'on ground' (Fig. 1). This means that the 'slow component' of X-ray-induced DNA repair, namely excision repair caused by base damage and inhibited by ara-C is not affected by simu-

Table I – Frequency of chromosome aberrations induced by X rays under microgravity conditions (Clinostat) and on ground in G_0 human Lymphocytes'

Treatment	Metaph. scored	Aberrations per 100 cells: Dic. and Rings (+F")	Aberrations per 100 cells: Excess Fragments	Total aberrations (%)	Aberrant cells (%)	Statistical signific.
Ground:						
Untreated	100	0.0	1.0	1.0	1.0	-
ara-C 5×10^{-5}M	100	1.0	5.0	6.0	6.0	N.S.
1.5 Gy	100	17.0	17.0	34.0	24.0	(1)***
1.5 Gy + ara-C	100	26.0	31.0	57.0	43.0	(2)***
Clinostat:						
Untreated	100	0.0	0.0	0.0	0.0	-
ara-C 5×10^{-5}M	100	1.0	2.0	3.0	3.0	N.S.
1.5 Gy	100	42.0	21.0	63.0	47.0	(1)***
1.5 Gy + ara-C	100	65.0	25.0	90.0	68.0	(2)***

Keys:
F": Fragment associated.
NS: Not significant
***: Significant versus relevant controls at $P < 0.001$ with Fisher's Exact Test when considering aberrant cells.
(1)***: Significant at $P < 0.001$ with Fisher's Exact Test when considering aberrant cells (1.5 Gy ground vs 1.5 Gy Clinostat).
(2)***: Significant at $P < 0.05$ with Fisher's Exact Test when considering aberrant cells (1.5 Gy + ara-C ground vs 1.5 Gy + ara-C Clinostat).

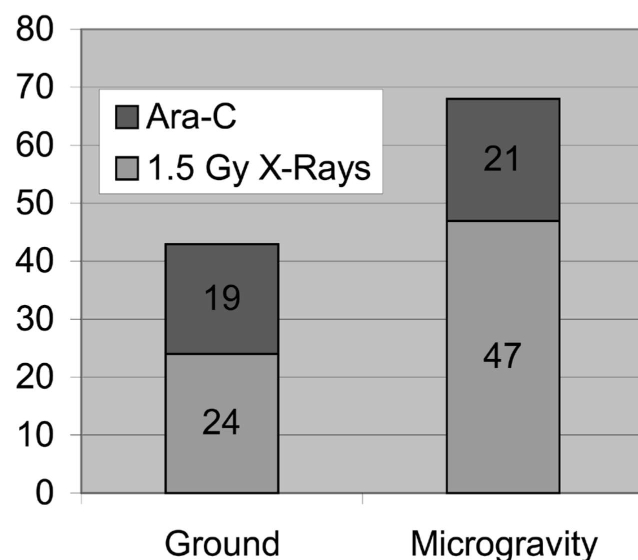

Fig. 1 – Frequency of aberrant cells induced by X-rays and ara-C in human lymphocytes in G0 under 'on ground' and simulated microgravity condition (clinostat).

lated microgravity.

On the contrary, what is instead affected is the 'fast component' of DNA repair, namely DNA double strand breaks (DSB) and single strand breaks (SSB) which are causing chromosome-type aberrations immediately after irradiation through mis-repair or mis-rejoining of DSB (Fig. 1).

The results obtained extend and corroborate previous findings obtained with the radiomimetic compound Bleomycin.

In conclusion we can state that:

• Under the reported experimental conditions, simulated microgravity (Clinostat) synergistically influenced the outcome of X-ray-induced chromosome aberrations.

• The 'slow component' of DNA repair (excision repair) inhibited by ara-C was not influenced by simulated microgravity.

A possible explanation for this outcome could quote two major factors:

• Enhanced probability that under simulated microgravity conditions the reactive DSB are spatially brought together to better interact, hence increasing the probability of mis-rejoining. This view could be supported by evidence that in the clinostat the exchange-type aberrations (dicentrics and rings) are more easily formed than fragments compared to the parallel treatments performed 'on ground'.

• Alternatively chromatin structure could be modified under simulated microgravity conditions generating different qualities and quantities of DNA lesions compared to treatments performed 'on ground'. This last aspect will be the object of next investigations.

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