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Exposure of Human Lymphocytes and Lymphoblastoid Cells to Simulated Microgravity Strongly Affects Energy Metabolism and DNA Repair

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Abstract Exposure of freshly drawn lymphocytes and lymphoblastoid cells (LB and COR3) to simulated microgravity decreased the intracellular ATP concentration to 50%–40% of the value found in normal growth conditions. The decrease was reversible although recovery to normal values occurred only slowly both in lymphocytes and in lymphoblastoid cells. Poly(ADP-ribose) polymerase (PARP) activity was increased indicating that cells exposed to conditions of reduced gravitation experience stress. Exposure to microgravity forces cells into a condition of metabolic quiescence in which they appear to be particularly sensitive to subsequent exposures to a genotoxic agent. Thus, treatment of cells with the strong redox agent potassium bromate under microgravity conditions, indicated an impairment in repair of DNA 8-hydroxy-2′-deoxyguanosine (8-OHdG), an oxidized derivative of deoxyguanosine. We conclude that gravitational modulation of the kind routinely obtained under laboratory conditions and during spaceflights is a stressful process to which cells appear to be extremely sensitive. These effects may reflect the physiological alterations observed in astronauts and in animals following spaceflights or exposure to conditions of simulated microgravity. J. Cell. Biochem. 94: 460–469, 2005. © 2004 Wiley-Liss, Inc.

Key words: microgravity; energy metabolism; DNA repair; 8-hydroxy-2′-deoxyguanosine; apoptosis

The modification or the loss of the gravitational force vector strongly affects many fundamental cellular functions [Hammond et al., 2000]. Continuous exposure to microgravity during spaceflights inhibits mitogenic activation and alters immunological parameters in lymphocytes [Cogoli, 1993; Pippia et al., 1996], inhibits proliferation and differentiation of haematopoietic progenitor cells [Davis et al., 1996] and modifies signal transduction processes in the leukemic myelomonocytic cell line U937 [Hatton et al., 1999]. An altered expression of the fas antigen and an increased susceptibility to apoptosis along with aberrant assembly of elements of the microtubular skeleton were seen in Jurkat cells flown on the space shuttle [Lewis et al., 1998]. The modulation of gravity should therefore be considered a powerful tool in the investigation of important physiological processes.

The effect of microgravity on gene expression has also been well documented using the microarray technology. In the study reported by Lewis et al. [2001], a set of 4,324 mRNAs were analysed after a 24 h exposure of Jurkat cells to microgravity on the Space Transportation System 95. More than 20,000 genes and expressed sequence tags (EST) were analysed on the same mission in an experiment performed with a 48 h exposure to microgravity. Expression of 11 cytoskeleton related genes including a centriole associated protein were up-regulated

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in space flown cells compared to ground controls. Significantly, changes in expression of genes encoding various proteins involved in the regulation of cell growth, metabolism, signal transduction, transcription, apoptosis, and tumour suppression were also observed [Walther et al., 1998; Batkai et al., 1999; Hatton et al., 2002; Sundaresan et al., 2002]. Similar changes in gene expression were reported in ground microgravity simulations employing dedicated instrumentations [Hashemi et al., 1999; Schwarzenberg et al., 1999; Walther et al., 1999].

In addition to changes related to gravity alone, it is also important to understand whether differential, and potentially cumulative, biological effects may derive from the concomitant exposure to cosmic radiations in spaceflight. This issue has been addressed in a review by Kiefer and Pross [1999].

We examined the effects on basal metabolism in freshly prepared human lymphocytes and the human lymphoblastoid cell lines LB and COR3 of exposure to simulated microgravity in a random positioning machine (RPM). In addition we studied the possible consequence of these alterations on cellular proliferation, apoptotic response, energy metabolism, susceptibility to DNA damage, DNA repair and modulation of poly(ADP-ribose) polymerase (PARP) activity.

A methodological issue related to the experimental schedule for exposure of lymphocytes to microgravity was also investigated. In a commonly accepted procedure, lymphocytes are activated by Concanavalin A (ConA), or other equivalent mitogens, simultaneously with exposure of the cells to microgravity [Cogoli et al., 1984]. This results in a strong inhibition of lymphocyte activation as shown by the dramatic reduction in the expression of the surface antigens CD25 and CD69 and by the absence of RNA synthesis. Inhibition of signal transduction is reflected in the early and strong perturbations in the integrity of various cytoskeletal components [Hashemi et al., 1999] resulting in an abortive proliferative response. In our opinion, the concomitant presence of these two opposite stimuli, microgravity and mitogens, may mask some of the effects of microgravity alone on normally proliferating cells. In our experiments, lymphocytes were activated well before exposure to microgravity in order to highlight any transient modifications in cellular metabolism due to the change in gravitational conditions. We believe that this experimental approach creates conditions much closer to the exposure to microgravity experienced by astronauts during spaceflights.

MATERIALS AND METHODS

Cell Lines and Treatments

PBL were purified from buffy coats from normal human volunteers. Samples were diluted in HBSS buffer and separated with Ficoll (Sigma Chem. Co., Milan, Italy). According to the manufacturer's protocol the purified cell population consists of >87% T-lymphocytes. After washing with PBS the cells were counted and resuspended in DMEM medium supplemented with 10% FCS, 25 mM Hepes 2 mM glutamine, and 50 U/ml penicillin/streptomycin and maintained at 37°C in 5% CO_2. Cell cycle was induced by activation with 15 μg/ml ConA and cells are maintained at standard growth condition for 18 or 24 h to reach, respectively, the S- or G_2-phase of the cells cycle before exposure to microgravity in the RPM.

Lymphoblastoid cell lines LB and COR3 are normal human lymphocytes respectively of the B and T lineage, immortalised by EBV infection. These cell lines were maintained in culture as reported above for lymphocytes. The growth and the proliferative ability of the cells were followed during the various phases of the experiment by the BrdU labelling and detection kit III (Roche Diagnostics, Milan, Italy). Cell viability was determined by trypan blue dye exclusion test. Cell proliferation was quantified through the determination of the cumulative population doubling level (PDL) calculated as log2 (D/D0) where D is the density of cells when harvesting and D0 is the density of cells when seeding. Exposure to microgravity was accomplished with a RPM (Dutch Space, Leiden, NL) located in a temperature controlled room at the University of Sassari. This instrument consists of two independently rotating frames. The spinning velocity of the frames was set up at 60°s-1. The separate rotation of each frame is random and driven by a dedicated software in order to obtain a constant simulation at the value of the g vector as low as chosen accordingly to manufacturer's instructions. The RPM is suitable to accommodate samples of different size and weight up to more than 10 kg. Routine conditions employed in our experiments were set for a value of 0.003 g.

DNA damage was induced by a 30 min treatment with 10 mM $KBrO_3$ dissolved directly in the growth medium at 37°C. KBrO3 is a mutagen able to induce oxidative DNA modifications with an high preferential formation of the adduct 8-deoxyguanosine (8-OHdG) over other type of base damages [Parsons and Chipman, 2000]. After the treatment, cells were washed with PBS and resuspended in fresh medium. $KBrO_3$ does not interfere with cellular proliferative ability and does not induce apoptosis in the conditions employed in this study.

Evaluation of Apoptosis

Human sFas ELISA Kit (BD Biosciences Pharmingen, San Diego CA) (Cat. Number 557071) was used for the quantitative determination of Fas released in the cell-free colture medium. Accordingly to manufacturer's instructions a standard curve for sFas was constructed for comparison with the content of the sFas measured in the aliquots of the cell colture medium sampled during the experiment. The concentration of sFas (U/ml) calculated in the assay was normalised for the cell number in the respective sample.

Quantification of ATP

Intracellular ATP quantification was achieved using the procedure described in the Sigma ATP reaction kit 366-UV protocol. Briefly, phosphoglycerate phosphokinase and glyceraldehyde phosphate dehydrogenase were employed for the conversion of ATP to ADP, concomitantly associated with the NADH oxidation to NAD. By determining the absorbance decrease at 340 nm a measurement of the original ATP content was obtained.

Quantification of PARP

PARP activity was determined by quantification of the incorporation of labelled ADP-ribose from (^{32}P)NAD (5 μCi/nmol) into acid insoluble material. PARP activity was expressed as pmol (min μg DNA)$^{-1}$. The total level of the catalytic protein was evaluated by autoradiography in cell extracts [Cesarone et al., 2000].

Western Blotting

Cell extracts were prepared according to Harlow and Lane [1988]. Extracts were maintained at −80°C until analysis and run according to Ottaggio et al. [2000]. HSP 70 and HSC 70 (the inducible, sc-1060, and constitutive, sc-1070, forms of the heat shock protein, respectively), and GRP 94 (sc-1794) antibodies against human antigens were used (Santa Cruz Biothecnology, Inc., Santa Cruz, CA). In test experiments HSP 70 was induced by the treatment of cells at 42°C for 30 min. GRP94 was induced by treatment with 10 mM 2-deoxyglucose in colture medium for 6 h. Actin (sc-1615) was used as a standard. Secondary antibodies were HRP-labelled and detection was accomplished with ECL.

Single Cell Gel Electrophoresis (SCGE) Assay

The SCGE assay was employed for the evaluation of DNA damage induced by the microgravity treatment itself and as an index of the condition of stress at which cells were subjected during the various phases of the study. SCGE was performed as previously described [Zunino et al., 2001]. Briefly, cells were suspended in 1% low melting point agarose in PBS at pH 7.4 and stratified onto a fully frosted microscope slide pre-coated with a layer of normal melting agarose. After 5 min on ice, slides were immersed in lysis solution (2.5M NaCl, 100 mM Na2EDTA, 10 mM Tris, pH 10, 1% Triton X-100) at 4°C for at least 1 h. Slides were than subjected to electrophoresis, washed and stained with ethidium bromide. Slides were scored under an Axioplan microscope (Zeiss, Oberkoken, Germany) equipped with fluorescence and then photographed. Comet lengths were measured on negatives using a Peak Scale Lupe 7X. At least two experiments were run for each condition.

Quantification of 8-OHdG

Damage induced by $KBrO_3$ treatment was quantified in the DNA of the various cell lines used according to the procedures reported for DNA extraction, purification, enzyme hydrolysis and HPLC/EC analysis [Zunino et al., 2001].

Statistical Analysis

Data were analysed by one-way ANOVA and unpaired two-tail Student's t-test using InStat software for McIntosh. The data presented are from at least three independent experiments and error bars are the standard error of the mean from duplicate samples.

RESULTS

For the evaluation of cell growth and viability three sets of flasks for each cell line were seeded

at a density of approximately 1×106 cells/ml and maintained in standard growth conditions until the beginning of the experiment. Two flasks for each lymphoblastoid cell line were filled with culture medium to avoid air bubbles and foam formation when mounted in the RPM. One flask was then mounted on the RPM and the second served as a control. The third set of flasks were maintained in standard growth conditions throughout. Cells were exposed to microgravity for 8 or 24 h. At the end of the exposure period, cells were returned to the incubators and allowed to recover under normal growth conditions for a further 24 or 48 h.

Figure 1 shows cell growth (panel A). Each determination of cell proliferation (cell doubling PDL) was accompanied by a measure of cell viability (panel B). At 24 h after treatment, there was a significant reduction in the PDL of LB and COR3 cells. A similar, but reduced effect on PDL was seen in lymphocytes at 8 h. This reduced cell proliferation was not due to microgravity [Lewis et al., 1998] since a similar decrease was found in unexposed flasks maintained in the same experimental conditions. This effect is most likely due to the stressful culture conditions during the experiment, maybe the limitation of nutrients and the growth factors exhaustion, and in particular to the inadequate exchange of gasses. As a parameter of cellular stress during the different phases of the experiment, we examined the release of sFas into the culture medium (Fig. 1, panel C). Maximal release of sFas was observed for samples collected at 24 h (8 h for lymphocytes). During the recovery time, normal cell growth conditions were restored and no further release of sFas was observed.

To determine whether DNA damage might have been induced by our experimental conditions we performed SCGE assays. DNA damage was found during each phase of the experiment (Fig. 2) but in general, the level was low. It appears that exposure to microgravity is not significantly associated with the induction of DNA damage.

ATP

Basal levels of intracellular ATP in lymphocytes, LB and COR3 cells were 3.87 ± 0.86, 2.23 ± 0.34 and 2.76 ± 0.25 μmol/106 cells, respectively. In control samples—either mock RPM exposed or maintained under normal growth conditions—there were no significant fluctuations in the level of basal intracellular ATP during the various phases of the experiment (Fig. 3). Exposure to microgravity induced a significant decrease in intracellular ATP. Figure 2 shows the kinetics of recovery of intracellular ATP over 48 h. Recovery to the basal ATP level is slow. Treatment with $KBrO_3$ did not significantly modify the intracellular ATP concentration (data not shown).

PARP

PARP activity is a crude indicator of a cell's general health [Burkle, 2000]. PARP participates in several processes involved in DNA protection. It contributes to genomic stability and to regulation of gene expression [Burkle, 2001]. An inability to modulate PARP activity results in genomic instability and enhanced susceptibility to DNA damage. PARP activity is particularly sensitive to environmental conditions of cell growth and can change very rapidly in response to changes. The response of the three cell lines employed in this study was very similar. Basal PARP activity was 0.25 ± 0.03 pmol/min/μg DNA for lymphocytes, 0.31 ± 0.18 and 0.27 ± 0.041 pmol/min/μg DNA for LB and COR3 cells, respectively. No changes in PARP activity were observed immediately after exposure to microgravity (Fig. 4). In LB and COR3 cells exposed to microgravity for 24 h and in lymphocytes for 8 h, PARP activity was increased 2-fold at 4 h from the end of the treatment. In lymphocytes the increase was 2.15 ± 0.39-fold while LB and COR3 cells showed 2.58 ± 0.76 and 1.84 ± 0.82-fold increases, respectively. By 24 h after the end of the exposure, PARP activity had returned to the level in unexposed cells. In conclusion, it appears that exposure to microgravity induces an increase in PARP activity and that this increase occurs some time after the end of the exposure.

PARP activity is affected by the exposure to $KBrO_3$. Treatment with $KBrO_3$ induced an increase of up to 1.5-fold in PARP activity in the samples exposed to microgravity and in controls (Fig. 5). In both microgravity-treated and control cells, basal PARP levels returned to normal 24 h after $KBrO_3$ treatment.

Western Blot

Cell extracts were prepared just after the exposure to microgravity in the exposed

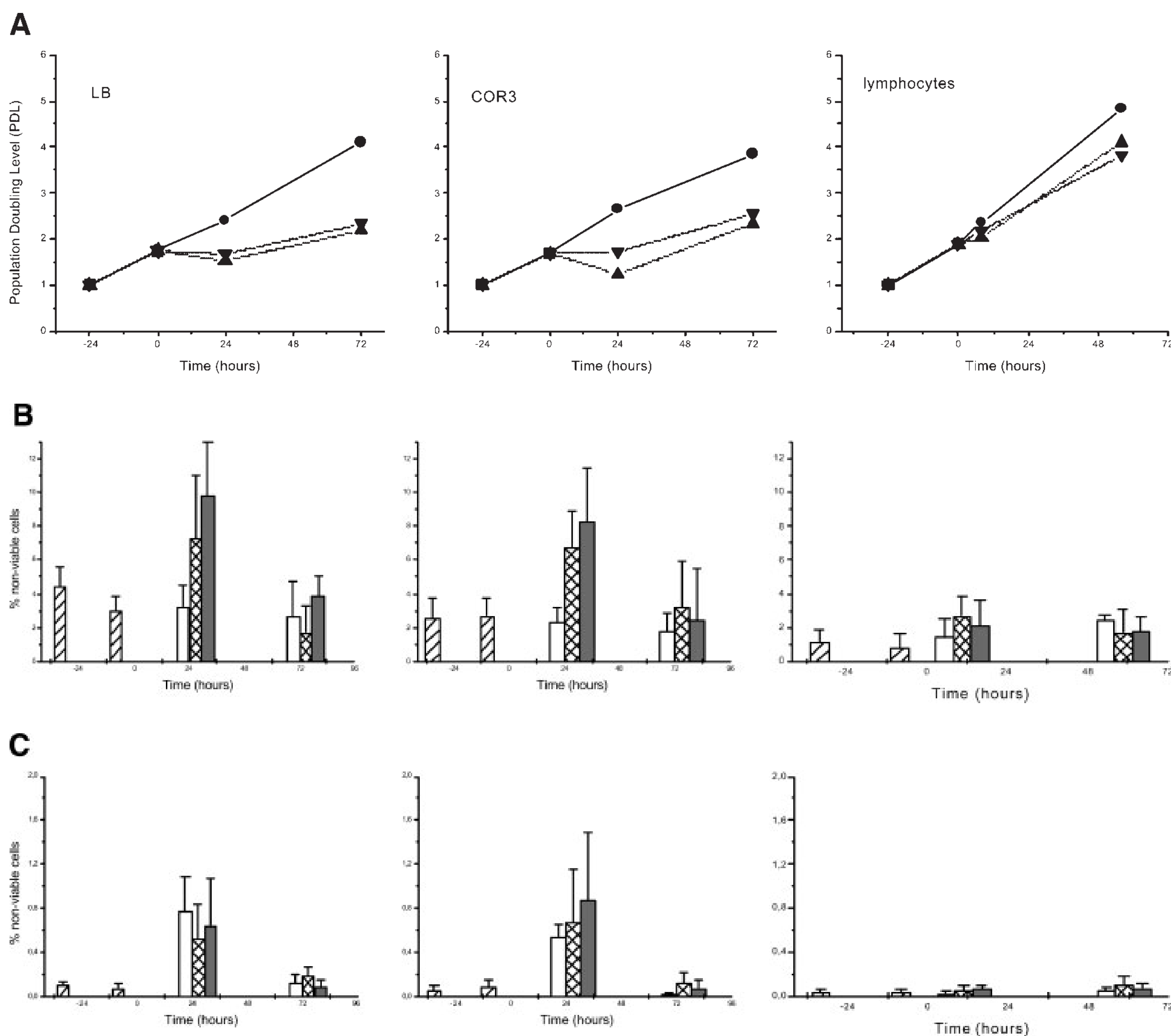

Fig. 1. **Panel A**: Cellular growth represented in function of the population doubling level (PDL) for LB, COR3 and normal lymphocytes is reported during the experimental time course. Cells are maintained in standard condition (from −24 to 0 h) until three differents aliquots are prepared. Exposure to microgravity is accomplished for 24 h (from 0 to 24 h, in figure) for LB and COR3 cells and 8 h (from 0 to 8) for lymphocytes. Growth of cells maintained as controls is reported as a straight line; growth of cells exposed to microgravity is reported with the dotted line and paired samples left unexposed are represented with the dashed line. **Panel B**: The fraction of non-viable cells during the various phases of the experiment is calculated after the trypan blue assay. Each experimental determination is matched with its the relative sample as shown on Panel A. The fraction of non-viable cells at time −24 and 0 h in the various cell lines is reported with the dashed columns. Fractions of non-viable cells for controls (white columns), microgravity exposed cells (grey columns) and control-matched microgravity-exposed cells (cross-dashed columns) are reported for the aliquots of cells taken at time point 24 and 72 h for the LB and COR3 cells or at 8 and 56 h for lymphocytes. **Panel C**: Quantification of sFas protein released into the cell colture medium during the various phases of the experiment. According to data presented on Panel B each experimental determination shown here matches with its the relative sample in Panel A. The values of sFas quantified at the time points −24 and 0 h in the various cell lines are reported as the dashed columns, far each cell line. Values of sFas released in controls (white columns), microgravity exposed cells (grey columns) and control-matched microgravity-exposed cells (cross-dashed columns) are reported for the cells taken at time point 24 and 72 h for the LB and COR3 cells and 8 and 56 h for lymphocytes. Data are mean of at least three independent experiments. Error bars represent SD of the mean.

samples and compared to matched unexposed controls. No differences in the expression of HSC70, HSP70 and GRP94 proteins were found.

DNA Damage

We examined whether microgravity induced an oxidative stress. No significant difference

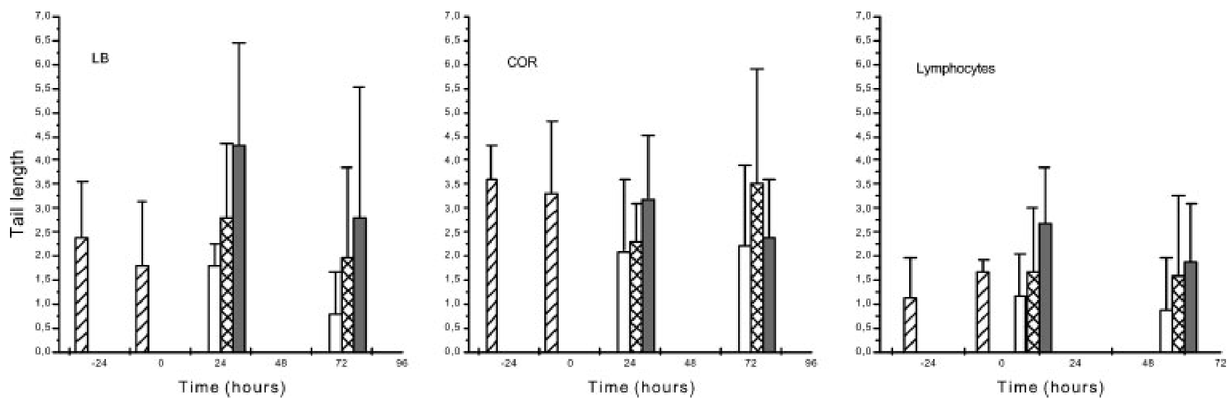

Fig. 2. Single cell gel electrophoresis (SCGE) assay's quantification of the DNA damage occurring in LB, COR3 and normal lymphocytes during the various phases of the experiment. Aliquots of cells are taken at −24 and 0 h (dashed columns), before handling of the cells. Aliquots were subsequently taken in samples maintained as controls (white columns), in microgravity exposed samples (grey columns) and in the control-matched microgravity-exposed samples (cross-dashed columns). Data are mean of at least three independent experiments. Error bars represent SD of the mean.

in the basal content of DNA 8-OHdG was found in controls or cells exposed to microgravity (Table I). These data indicate that microgravity does not induce a measurable increase in the content of DNA 8-OHdG.

Cells were treated with KBrO3 to induce a significantly elevated level of 8-OHdG in DNA (Table I). Following damage induction by $KBrO_3$, the kinetics of removal of DNA 8-OHdG was studied. No significant difference was apparent in damage induction between cells exposed or unexposed to microgravity (Table I). As shown in Figure 6, LB and COR3 cells and lymphocytes that were not exposed to microgravity all efficiently removed 8-OHdG from their DNA with half-lives of 1.34 ± 0.21 h, 1.23 ± 0.13 h and 1.26 ± 0.12 h, respectively. After exposure to microgravity DNA repair was strongly decreased. Removal of 50% of DNA 8-OHdG occurred in 14.42 h in lymphocytes, and in 8.12 and 5.54 h for LB and COR3 cells, respectively.

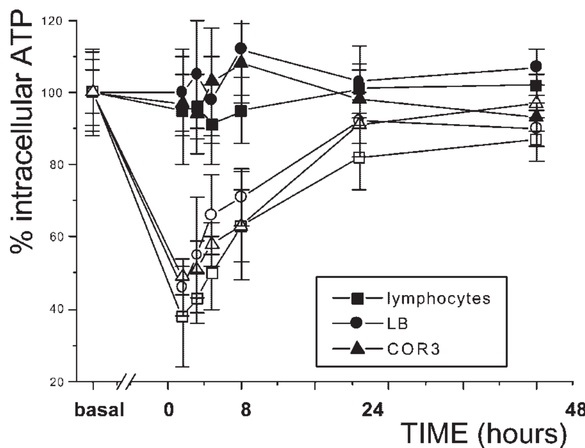

Fig. 3. Quantification of the intracellular ATP content for the different cell lines employed in the study. The measured ATP values are reported as percentages with reference to the basal level measured for each cell line maintained in normal growth conditions. The abscissa reports the time course from 0 to 48 h (from 72 to 120 h in the experiment) of the exposure to microgravity, in the recovery phase, for cells exposed to microgravity and also for those maintained unexposed as ground controls. Squares are representative of data for lymphocytes, circles for LB cells and triangles for COR3 cells. Solid symbols are for samples maintained in ground conditions and the open symbols are for the samples exposed to microgravity. Data are mean of at least three independent experiments. Error bars represent SD of the mean.

DISCUSSION

Microgravity does not appear to significantly affect cellular survival or to induce DNA damage. Apoptosis induction, release of sFas and fluctuation of PARP activity occur transiently and only to a minor extent. Expression of HSPs or GRPs is also unaffected by microgravity. Conversely cells exposed to microgravity display a dramatic decrease in the intracellular concentration of ATP. This decrease in intracellular ATP may be due to a diminished activity of mitochondria. Switching off mitochondrial metabolism, which results in a proliferative arrest, represents a means of escape from cell death. This mechanism might explain the absence of activation of the stress response associated with mitochondrial damage. Taken together with the diminished proliferation rate, these findings suggest that microgravity exposure induces a condition of metabolic 'quiescence'.

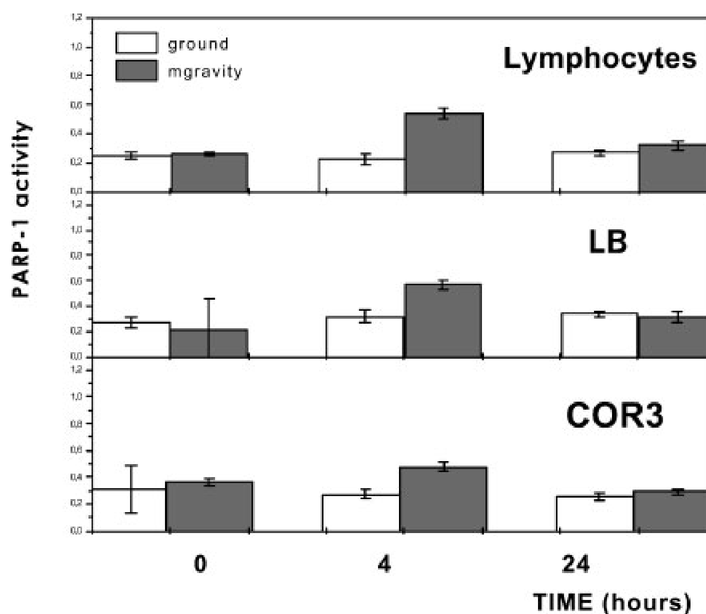

Fig. 4. Fluctuation of the poly(ADP-ribose) polymerase (PARP) activity during the various phases of the experiment for the three cell lines employed. PARP activity is expressed as pmol/min/μg DNA. PARP was measured at time 72 (0 h in the figure), just after the exposure to microgravity, and 4 and 48 h later (at 76 and 120 h, respectively), during the recovery phase, for the cells exposed to microgravity as well for those maintained unexposed as ground controls. Data are representative of at least three independent experiments except for data obtained from lymphocytes that were performed only twice. Error bars indicate SD of the mean.

Cells do adapt to changes in their environment and respond to the absence of gravity with alterations at the biochemical and molecular level. Indeed structural modifications of microtubules and cytoskeleton have been reported in cells exposed to simulated microgravity on earth and in experiments performed in space [Lewis et al., 1998]. Modifications in cell growth,

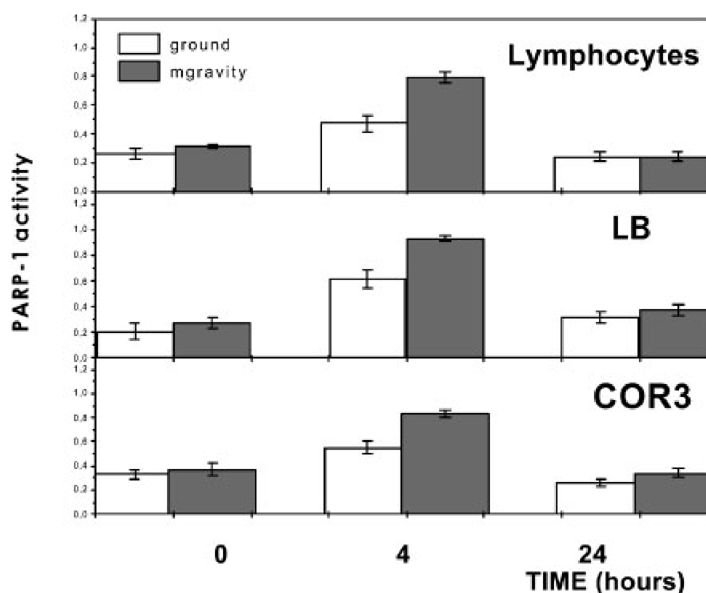

Fig. 5. Fluctuation of the PARP activity after exposure of cells to $KBrO_3$. PARP was measured at time 72 h (0 h in the figure), just after the exposure to microgravity and the treatment with $KBrO_3$, 4 and 24 h later (at 76 and 120 h, respectively), during the recovery phase, in cells exposed to microgravity and in the ground controls. Data (±SD reported as error bars) are representative of at least three independent experiments except for data obtained from lymphocytes that were performed only twice.

TABLE I. 8-OHdG Quantification During the Various Phases of the Experiment

	8-OHdG	(mol 8-OHdG/10^6 mol dG)		
	−	−	+	+
$KbrO_3$ microgravity	−	+	−	+
Lymphocytes	0.53 ± 0.32	0.49 ± 0.27	1.96 ± 0.16*	2.23 ± 0.21*
LB	0.61 ± 0.26	0.67 ± 0.31	2.40 ± 0.43*	2.11 ± 0.24*
COR3	0.87 ± 0.25	0.83 ± 0.18	3.24 ± 0.36*	2.84 ± 0.34*

*$P < 0.001$.
Quantification of 8-OHdG after microgravity exposure was at 72 h.
Quantification after treatment with $KbrO_3$ was performed just after the exposure to the drug.
Data represent mean ± SD of at least three independent quantifications.

in immunological properties and in different cellular activities have also been reported [Cogoli, 1993]. A decrease in intracellular ATP has been correlated with problems related to cell cycle progression [Sweet and Singh, 1999] and limitations in ATP availability typically induce a G_1 or G_2-M cell cycle arrest [Sweet and Singh, 1999].

The changes we identified after the exposure of cells to microgravity are transient and reversible. Twenty-four hours after the end of the exposure to microgravity all the parameters we examined had returned to pretreatment values. This behaviour is shared in lymphocytes and immortal lymphoblastoid cells. If enough time is given to cells to restore initial conditions this would result in an undetectable condition of risk.

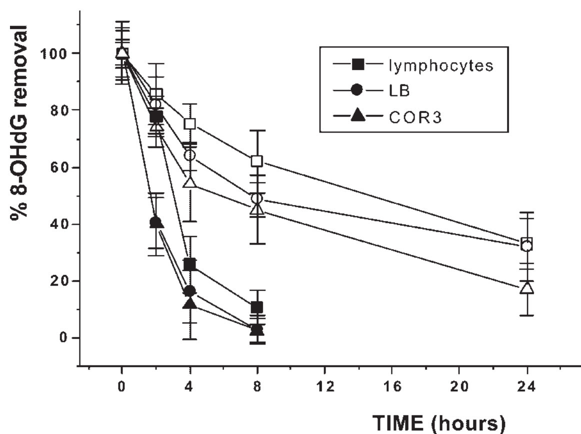

Fig. 6. Time course for removal of 8-OHdG from DNA in the different cell lines employed in the study after treatment with $KBrO_3$. Squares represents data from lymphocytes, circles for LB cells and triangles for COR3 cells. Solid symbols are for the 8-OHdG removal in controls from microgravity-unexposed $KBrO_3$-treated samples. Open symbols are for the samples exposed to microgravity and treated with $KBrO_3$. Data (±SD reported as error bars) are representative of at least three independent experiments.

Published data show that the consequences of exposure to microgravity are visible as soon as few minutes after the beginning of the exposure [Schatten et al., 2001; Uva et al., 2002; Maccarrone et al., 2003]. Upon exposure to microgravity cells undergo physical and behavioural changes through a fast and chaotic adaptation phase. Finally cells reach a state of equilibrium in which novel structural and metabolic characteristics are maintained. At the cessation of the exposure, when cells are returned to normal growth conditions, functions are restored to the previous conditions. Many cell activities related to the maintenance of the cell's health are involved in this adaptation process, as evidenced by the large number of alterations in gene expression identified in microarray experiments [Lewis et al., 2001]. However, these precocious and early responses are transient and the prestress conditions are recovered in a short time after the end of the stimulus.

Studies performed employing entire organisms confirm these observations where the adaptation to microgravity or hyper-gravity is accompanied by the same behavioural modifications observed in plants, seeds and animals [Hilbig et al., 2002]. When the animal has adapted to an apparently normal behaviour in the modified environmental conditions, reestablishment of the original conditions is accompanied by the same reactions observed in the occurrence of the first alteration. Which are the steps involved in the adaptation to the new environmental conditions and the risks associated to these processes is still unknown. However these steps take time. Since these

processes may reflect the physical and clinical alterations found in astronauts and animals who experienced spaceflights or conditions of simulated microgravity this problem is worth to be studied.

In our hands when $KBrO_3$ was administered to cells just at the end of the exposure to microgravity, a dramatic loss in the ability of the cells to counteract the induced DNA damage was identified. The extent of DNA damage induced by $KBrO_3$ in cells exposed to microgravity did not differ from that induced in controls. This implies that the cellular redox metabolism and the GSH content are not affected directly by the microgravity exposure and, as these cells efficiently modulate the PARP response, also the intracellular NAD should not be depleted. However, the dramatic change observed in the kinetics of DNA 8-OHdG repair in these conditions suggests that microgravity induces a condition of exceptional vulnerability of the cells to external agents. DNA repair kinetics results significantly slackened and complete repair requires more than 24 h. The decrease in the efficiency of the DNA repair process can vary from 5- to 10-fold depending on the cell line. It is interesting to note also that the process appears strictly related to the recovery of the intracellular ATP level to the conditions of unexposed cells. Treatment with $KBrO_3$ makes clear how a DNA insult delivered during the narrow time window in which the cells did not yet restore the basal metabolic parameters to the pre-stress conditions, may have relevant consequences for the future health status of the cells. By extrapolation we suspect that the same problem might apply to animals and humans experiencing micro-gravitational stress in laboratory conditions or in spaceflights. The knowledge of the physiological state of the cells during microgravity exposure and the characteristics of their alterations might be very important in the development of suitable interventions and counter measures to avoid harmful effects induced by this exposure.

The use of simulated microgravity might allow to understand if differential and potentially cumulative effects may derive from the concomitant exposure to cosmic radiations in spaceflights. Recent data upon this issue have been reviewed [Kiefer and Pross, 1999]. Although in the published data DNA repair processes do not appear to be altered by microgravity exposures, our data indicate that this conclusion cannot be considered a general one since we showed that the DNA repair process, is modified in its kinetics. Allowing enough time for the process to be completed no hint would indicate a difference in the execution of DNA repair.

Several authors [Le Bourg, 1999; Wang, 1999] reported a similarity between the phenotypical changes occurring after spaceflight and those reported in the process of ageing of cells and tissues. Some of the results reported by us may well be considered in the same way as the ageing process proceeds through a decrease in cellular proliferation, in energy production, or in the adaptation to changing environmental conditions. In this context we might consider microgravity as a model to study processes associated with ageing or some of the degenerative processes typically associated with senescence. In addition some of the effects induced by microgravity on the assembly of the microtubules and on cytoskeleton elements are typical of a defective chromosomal segregation which underlies the carcinogenic processes. It will be of interest to study the behaviour of tumour cell lines in the same experimental conditions to verify whether the process of neoplastic transformation alter the response to microgravity [Degan et al., 2001].

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