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Analysis of spontaneous and bleomycin-induced chromosome damage in peripheral lymphocytes of long-haul aircrew members from Argentina

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Abstract

Spontaneous and bleomycin (BLM)-induced chromosomal aberrations in G0 and G2 stages of the cell cycle have been analyzed in peripheral lymphocytes of 21 long-haul aircrew members from Argentina in order to assess BLM-induced clastogenesis as a first approach to determine the DNA repair capacity and thereby the susceptibility to environmental cancers in aircrew. The possibility that occupational exposure of flight personnel to cosmic radiation can induce an adaptive response in their peripheral lymphocytes that can be detected by a subsequent *in vitro* treatment with BLM was also investigated. For comparison, aberrations were also scored in the lymphocytes of 15 healthy volunteers matched by age, health, sex, drinking and smoking habits to the flight personnel group. Aircrew exhibited a higher frequency of spontaneous dicentrics and ring chromosomes than the control population ($p < 0.05$). BLM sensitivity test showed that aircrew and controls are equally sensitive to BLM G2 clastogenic effects, since both groups exhibited a similar frequency of chromatid breaks per cell ($p > 0.05$). However, the aircrew sampled population was almost two times more sensitive to BLM G0 clastogenic effects than controls ($p < 0.05$). Therefore, our data suggest that chronic exposure of aircrew to cosmic radiation increases the *in vitro* chromosomal sensitivity of their peripheral lymphocytes to BLM (at least in the G0 stage of the cell cycle), and that occupational exposure of flight personnel to cosmic radiation does not induce an adaptive response to this radiomimetic compound. Our results justify further studies aimed at determine if those aircrew members hypersensitive to BLM are more prone to develop environmental cancer than BLM-insensitive individuals.

Keywords: Aircrew; Chromosome damage; Bleomycin; Mutagen sensitivity assay; Bleomycin assay

1. Introduction

Airline crew members on high altitude flights are occupationally exposed to elevated levels of ionizing radiation that are significantly higher than at ground level [1–7]. This radiation is primarily of cosmic origin and is largely composed of high-energy particles,

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predominately protons (see [6,7] for review). Secondary radiation is generated by energetic particles as they collide and interact with air nuclei and other intervening matter. These reactions mostly produce protons, neutrons, electrons, and gamma and X-rays. The neutrons contribute more than 50% of the effective radiation dose present at altitudes used for commercial flying [6]. Additional radiation exposure comes from solar cosmic radiation, which also consists of charged particles, largely protons, but the energy of these particles is usually lower than those of cosmic radiation [8–10]. Cosmic rays easily penetrate the body and, therefore, are a major concern in relation to the health of aircrew and other occupationally exposed workers such as couriers, and the flying public, particularly frequent fliers.

The extent of radiation exposure in flight personnel depends on the routes, duration, flight altitude, latitude, and solar flares. On intercontinental routes, at flying cruising altitudes higher than 10,000 m, the estimated mean cumulative exposure for the aircrew members is on average 3 mSv/year, with a range from 1 to 10 mSv/year [4,6,7,11]. This dose is substantially larger than the annual limit of 1 mSv recommended by the International Commission for Radiological Protection (ICRP) for the general public [12]. Therefore, in 1990 the ICRP recommended that aircrew members should be regarded as occupationally exposed to ionizing radiation [12]. Aircrew members of international flights from Argentina make intercontinental flights with cruising altitudes of 10,000–13,000 m, and thus are highly exposed to cosmic radiation [13]. Therefore, they constitute a good sample to investigate the effects of cosmic radiation on flight personnel.

Since aircrew of international flights are chronically exposed to cosmic radiation and other environmental hazards, there have been some suggestions that commercial aviation flight personnel is at a higher-than-normal risk of developing cancer. Although more than 20 epidemiologic studies of cancer incidence and mortality in aircrew members of commercial flights have been published since 1990, there still is not a clear cause-and-effect relationship between risk of any site-specific cancer and employment as a pilot or flight attendant (see [7], for review). However, increased risks for malignant melanoma of the skin among both pilots and cabin crew, and female breast cancer among flight attendants have been relatively consistent findings (see for example, [14–17] and [7] for review).

It is well known that the endogenous sensitivity to DNA damage plays an important role in the development of cancer (i.e., the way in which an individual deals with DNA damage is related to the chance to develop cancer) [18–20]. Therefore, the analysis of the DNA repair capacity in aircrew is of medical interest. An indirect way to assess the DNA repair capacity is to determine the chromosomal sensitivity of peripheral blood lymphocytes to clastogens, i.e., chromosome breaking agents (the so-called mutagen sensitivity assays [20–23]). The most widely used mutagen sensitivity assay is the bleomycin (BLM) test [21,22]. BLM is an S-independent radiomimetic agent that induces double-strand breaks in DNA [24]. In the BLM *in vitro* sensitivity test, mutagen sensitivity is determined in peripheral blood lymphocytes as the mean number of chromatid breaks per cell (b/c) at metaphase induced by BLM exposure in the late S–G2 phase of the cell cycle [22,25–27]. Cells of persons deficient in DNA repair respond with high numbers of BLM-induced chromatid breaks. Therefore, a high number of BLM-induced b/c is strongly associated with the development of environmentally related cancers, such as colon cancer, lung cancer, and head and neck squamous cell carcinoma ([21,25,28]; see Refs. [22,23] for review). In the present work, we analyzed the chromosomal sensitivity to BLM in a sample of aircrew of international flights from Argentina in order to assess, for the first time, the risk of BLM-induced clastogenesis in flight personnel as a first approach to determine the DNA repair capacity, and thereby the susceptibility to cancer in aircrew. We also investigate if occupational exposure of flight personnel to cosmic radiation can induce an adaptive response (i.e., increases the effectiveness of the DNA repair system) in their peripheral lymphocytes that can be detected by a subsequent *in vitro* treatment with BLM. The adaptive response is a phenomenon in which cells exposed to low, non-genotoxic doses of a mutagen become less susceptible to cytogenetic damage by subsequent high doses of the same or another genotoxic agent [29–33]. A decreased sensitivity to the cytogenetic effects of BLM in individuals exposed to ionizing radiation (children contaminated as a consequence of the Chernobyl accident [34] and hospital workers from radiotherapy or radiodiagnostic services [35]) has been previously reported. Since aircrew members are exposed to low doses of ionizing radiation for years, an adaptive response phenomenon like that reported for other individuals exposed to radiation is likely to occur.

To accomplish the goals of this study, we analyzed the spontaneous and BLM-induced chromosome damage in the G0 and G2 (i.e., BLM sensitivity assay) stages of the cell cycle in peripheral blood lymphocytes from both aircrew and control, non-exposed individuals. We found that aircrew and controls are equally sensitive to BLM G2 clastogenic effects, but aircrew are almost two