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Neuroimmune response and sleep studies after whole body irradiation with high-LET particles

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Abstract

In order to investigate the biological effects of galactic rays on astronaut cerebral functions after space flight, mice were exposed to different heavy ions (HZE) in whole-body conditions at doses comparable to the galactic flux: ^{12}C, ^{16}O and ^{20}Ne (95 MeV/u, at 42–76 mGy). Animals were also exposed to 42 mGy of ^{60}Co radiation for comparison with HZE. The neuroimmune response, evaluated by interleukin-1 (IL-1) measurement, showed that this cytokine was produced 3 h after irradiation by ^{16}O or ^{60}Co. In contrast, neither ^{12}C (56.7 mGy) nor ^{20}Ne (76 mGy) induced IL-1 production. However, immunohistochemical staining of ^{12}C-irradiated mouse brain tissue showed 2 months later a marked inflammatory reaction in the hippocampus and a diffuse response in parenchyma. Sleep studies were realized before and after exposure to 42 mGy of ^{16}O and 76 mGy of ^{20}Ne: only the ^{20}Ne radiation displayed a small effect. A slight decrease in paradoxical sleep, corresponding to a reduction in the number of episodes of paradoxical sleep, was manifested between 8 and 22 days after exposure. Exposure to ^{12}C and ^{16}O induced no changes either in cellularity of spleen or thymus, or in caspase 3 activity (as much as four months after irradiation). Taken together, these data indicate that the CNS could be sensitive to heavy ions and that responses to HZE impact depend on the nature of the particle, the dose threshold and the time delay to develop biological processes. Differences in responses to different HZE highlight the complex biological phenomena to which astronauts are submitted during space flight.

Keywords: HZE particles; Brain; Neuroimmune system; Immune system

1. Introduction

The prospect of long-term space travel raises the question of astronaut safety. Among the potential risks in space, radiation exposure is a major one. Space flight entails a particular environment where many factors combine to influence a variety of physiological functions. These various aspects of the space environment can affect many physiological systems. Due to radiation exposure, impairment of central nervous system (CNS) function, with its potentially serious consequences, is a paramount concern. Furthermore, the combination of weightlessness, psychological stress and irradiation by solar particles events could exert synergistic effects on CNS function as well as on metabolism in general and on the immune system. Solar particle events are composed of mixtures of different high-energy charged particles (HZE), and during a major event, the flux of heavy, high-energy particles can make up 1% of the galactic cosmic radiation. Consequently, this

Abbreviations: LET, linear energy transfer; IL-1, Interleukin-1; HZE, high energy charged particles; CNS, central nervous system.

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suggests that the deleterious effects of exposure to these particles may be significant and cannot be ignored.

HZE can actually have significant effects on neuronal and behavioral functions. Exposure to HZE has been found to alter various behavior functions several months later in relation with neurochemical deficits (Joseph et al., 2000) that affect cognition (Hunt et al., 1989) and motor performance (Joseph et al., 2000). Moreover, at the level of brain tissue, lesions were found in the brain parenchyma of rats 15 days after 6–12 h of exposure to heavy cosmic ions (Pfister et al., 1976) with neuronal degeneration, and glial reactions (Nogues et al., 1978). Morphological modifications have been also observed in the olfactory bulb of mice 500 days after exposure to ^{60}Co, ^{12}C, ^{20}Ne, ^{56}Fe and ^{4}He (Kraft and Cox, 1986).

Among various factors of these central impairments, cytokine interleukine-1β (IL-1β) is a major one. IL-1β is produced in CNS following an inflammatory episode, in various brain regions, where it induces an inflammatory response with modification of sleep (Krueger et al., 2001), thermogenesis, and behavior (Dantzer et al., 1998). It is also implicated in lesion development (Tringali et al., 2000) consequent to the inflammatory event. 0042y contrast, IL-1β participates in the restoration of the biological functions by centrally-acting on a negative feedback loop controlling immune responsiveness (Sundar et al., 1989). Moreover, IL-1β is completely implicated in neuroimmune response during these episodes. An increasing amount of evidence indicates that there are bi-directional circuits between the CNS and the immune system which are mediated by similar soluble substances. The same cytokines and cytokine receptors are used for communications within and between the two systems and the neuroimmune reaction interferes with the control of central and peripheral endpoints. In fact, the peripheral, immune system is also very sensitive to HZE exposure. As previously described, the profile of leukocyte subsets, natural killer cell activity (Meehan et al., 1992, 1993; Stowe et al., 1999), and the production of interferon and other cytokines (Crucian et al., 2000) are immediately modified after the return of crews from space. Moreover, changes in leukocyte subsets, severe impairment of the capacities of bone marrow progenitor cells to respond and differentiate into mature immunocompetent cells (Sonnenfeld et al., 1990, 1992), and decreased interferon-γ production (Gould et al., 1987) have also been described in animals studies. Finally, low-dose radiation exposure with a high linear energy transfer (LET) particle, ^{12}C, activates the hypothalamo-pituitary–adrenal axis resulting by an increased in plasmatique corticosterone and ACTH, 6 h and 3 days after exposure (Lebaron-Jacobs et al., 2003).

Based on the neuroimmune actions, there is strong evidence to suggest that irradiation with different HZE particles, at doses previously used showing neuroendocrine activation, could compromise central functions like sleep and immunological reaction. Therefore, the purpose of this study was to evaluate the effect of various HZE particles, representative of the galactic flux, on IL-1β production in the brain (as a representation of the neuroimmune response) and to correlate it with paradoxical sleep (REM) and immunological endpoints.

2. Materials and methods

2.1. Animals

Animal experiments were conducted according to European Animal Care Commission guidelines (Ministry of agriculture act no. 87-848, October 19th 1987, as modified on May 29th 2001). Male Balb/C mice (20–25 g) were purchased from CER Janvier breeding facilities (France). Animals were given food and water *ad libitum*, and housed in a humidity- and temperature-controlled animal facility with a light–dark cycle.

2.2. Irradiation procedure

Mice were anesthetized with ketamine (Imalgene®, 150 mg/kg, i.p.). Groups of three mice were placed on a Plexiglas sheet and were whole-body irradiated through an irradiation-window of 4 cm × 20 cm, by ^{12}C, ^{16}O and ^{20}Ne beams. The beam energy delivered by the accelerator was 95 MeV/A. Due to the crossing of the 10 μm stainless steel window and 5 cm of air, the energy at the mice skin surface is slightly lower: 94.8, 94.7 and 94.6 MeV/A for, respectively, the ^{12}C, ^{16}O and ^{20}Ne beams. The beam flux was monitored by a plastic scintillator counter placed at one end of the Plexiglas sheet. Previously to the mice irradiation, the scintillator was calibrated by exposing CR39 plates (track detectors). The latent tracks induced in the CR 39 plates were chemically etched and counted with an optical microscope. This gave the relation between the fluence and the scintillator counting. The mice were irradiated at the following fluences (Φ): 1.15×10^6, 4.58×10^5 and 4.9×10^5 particles cm^{-2}, for the ^{12}C, ^{16}O and ^{20}Ne irradiations, respectively. The LET and mean track lengths were calculated in pure liquid water by the SRIM 2003 code. The ion mean ranges are 2.34 cm for ^{12}C beam, 1.75 cm for ^{16}O beam and 1.39 cm for ^{20}Ne beam. The mean dose delivered to the mice was calculated by the classical relation D (Gy) $= 1.6 \times 10^{-7} \times \Phi$ (cm^{-2}) $\times$ LET (MeV cm^2 mg^{-1}). As the mice were irradiated on the high energy side of the Bragg peak, the LET, and consequently the dose, increase with depth. If we consider the first centimetre depth, the average LET were 30.8, 56.8 and 967 keV/μm for ^{12}C, ^{16}O and ^{20}Ne beams, respectively. The corresponding averaged doses were 56.7 mGy (^{12}C), 41.7 mGy (^{16}O) and 76 mGy (^{20}Ne). Obviously, the dose variation with depth is smallest for the lightest projectiles. The ratio between the skin dose and the 1 cm averaged dose was 0.88, 0.86 and 0.74 for the ^{12}C, ^{16}O and ^{20}Ne beams, respectively. Thus, irradiation of mice was performed at on the left ascending side of the curve of the Bragg peak in order to avoid high variation of the dosimetry inside the brain.