Poster Session I

Prevention/Protection

7:00 p.m. – 9:00 p.m.
Ballroom I/II
Countermeasures for Space Radiation Induced Neoplastic Changes in Cells of Hematopoietic Origin and Radiation Induced Mortality

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INTRODUCTION

The hypothesis being evaluated in this research program is that control of radiation induced oxidative stress will reduce the risk of cancer development and radiation induced mortality in mice. As part of this grant work, we have evaluated the protective effects of several antioxidants and dietary supplements and observed that a mixture of antioxidants (AOX), containing SeM, N-acetyl cysteine (NAC), ascorbic acid, vitamin E succinate, coenzyme Q10 and alpha-lipoic acid, is highly effective at reducing space radiation induced oxidative stress in both in vivo and in vitro systems, and space radiation induced cytotoxicity and malignant transformation in vitro. Another antioxidant dietary supplement, Bowman-Birk Inhibitor Concentrate (BBIC), a soybean extract enriched in the protease inhibitor known as the Bowman-Birk inhibitor, has also been utilized in these studies.

METHODS AND RESULTS

The antioxidant dietary preparations described above (AOX and BBIC) are being evaluated as dietary countermeasures against space radiation induced myeloid leukemia in mice. Preliminary analysis of the data indicate that AOX and BBIC dietary supplements in the diet of male CBA mice exposed to radiation from either protons or iron ions can confer protection against neoplastic growth in cells of hematopoietic origin. The results of these studies are as follows: 1) Proton exposure (300 cGy) led to a significant increase in the development of malignant lymphoma (p<0.01). Mice irradiated with protons (300 cGy) and maintained on the AOX-supplemented or BBIC-supplemented diets had malignant lymphoma levels comparable to the control unirradiated mice maintained on the control diet (AIN-93G rodent diet). 2) Iron ion radiation exposure marginally increased the development of malignant lymphoma (p=0.05). Mice irradiated with iron ions (50 cGy) and maintained on the AOX-supplemented or BBIC-supplemented diets had malignant lymphoma levels comparable to the control unirradiated mice maintained on the control diet. 3) Proton exposure (300 cGy) led to a statistically significant increase in histopathologic lesions of myeloid origin; this category includes both myeloid hyperplasia in the spleen and myeloid leukemia. Mice irradiated with protons (300 cGy) and maintained on the AOX-supplemented or BBIC-supplemented diets had levels of histopathologic lesions of myeloid origin comparable to the unirradiated mice maintained on the control diet. 4) Iron ion exposure (50 cGy) did not lead to a significant increase in the levels of histopathologic lesions of myeloid origin.

In separate experiments performed during the past year, it has been observed that this mixture of antioxidant agents can reduce radiation induced mortality in ICR mice exposed to 8 Gy of x-ray irradiation. At 24 hours after an 8 Gy dose, it was observed that there was a significant protective effect of the AOX supplement on polymorphonuclear leukocytes (PMNs) and total white blood cells. At 24 hours after a dose of 1 Gy, peripheral blood cell counts indicate that AOX dietary supplementation can reduce radiation induced cell killing in total white blood cells, PMNs and lymphocytes.

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Amifostine modulates multiple toxicity of gamma-ray and fission-neutron exposures in mice

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Four thousand B6CF strain mice were subjected to gamma or fission-spectrum neutron radiation between October 1984 and October 1985, and treated either with saline or one of two radioprotectors. Two radioprotectors were Amifostine, S-2-(3-aminopropylamino) ethylphosphorothioic acid (WR2721) and phosphorothioate S-3-(3-methylaminopropylamino) propylphosphorothioic acid (WR151327); injected intraperitoneally thirty minutes before radiation exposure. Irradiation was administered in a single dose to animals 114 to 121 days of age. Ten distinct combinations of radiation type, dose, and radioprotector treatment were done on 200 male and female mice each. The presence of any of 155 specific gross pathological abnormalities and their lethality were recorded during autopsy. Stata 9/SE (StataCorp LP, TX) was used to analyze the impact of dose and radioprotector treatment on the genesis of these toxicities. Analysis compared the total number of toxicities found during autopsy in the mice of each radiation group to those found in the mice of the control group. In that analysis, the total number of toxicities per mouse was used as the continuous outcome variable. The number of toxicities per mouse ranged from one to fourteen. These cumulative-toxicity analyses were adjusted for age using dummy variables representing age at death quartiles, and the control group was used as the reference category. Multiple linear regression was used for analysis; all treatment groups of a specific radiation type were regressed in single model and significant differences from the control group were determined on the basis of the significant differences (p < .05) in the independent variable coefficients.

Using the total number of toxicities in each mouse as an outcome measure, linear regression revealed certain significant differences between the average number of toxicities in the control group and radiation groups. The model comparing the gamma-irradiated groups revealed that the 200cGy saline-injected (p < 0.001) and 400cGy Amifostine injected (p = 0.013) groups both had significantly higher numbers toxicities per mouse than controls when adjusting for age. In contrast, the 200cGy amifostine-treated group did not differ from the control group significantly (p = 0.19), suggesting that amifostine is, on average, protective against a number of lethal and non-lethal gamma-radiation-induced toxicities. All neutron-irradiated groups showed significantly more toxicities than the control group except the 10 cGy amifostine-treated group. The 10cGy saline-treated group on average had 0.32 more toxicities than the control group when adjusting for age (p < 0.01) while the amifostine and 10 cGy treated group did not have significantly more toxicities than the control group (p > 0.05). The 10cGy WR151327 and 40cGy amifostine- and WR151327- treated animals on average had .5 to .64 more toxicities than controls (p < 0.001). The insignificant difference between the low-dose amifostine group and controls, suggests that amifostine is protective against a number of lethal and non-lethal neutron-radiation-induced toxicities.

The cytoprotective and anti-mutagenic mechanisms of action amifostine are well established. Once in the cell the positively charged thiol and disulfide forms of amifostine or other phosphorothioates bind to the minor groove of DNA, stabilize damaged sites and facilitate their correct repair. Moreover, these agents delay cell cycle progression and allow for more time for the correct repair of DNA damage before it is fixed at mitosis. In addition, these studies show that at 200cGy gamma-rays and 10cGy neutron exposures amifostine completely protects mice from developing multiple non-lethal toxicities.
CBLB600s: a family of novel compounds with radioprotective and hematopoietic stem cells stimulating activity, acting via activation of TLR2 receptor complexes
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CBL is developing radiation countermeasures based on natural and artificial activators of Toll-like receptors. Lipoproteins from different bacteria stimulate cell surface TLR2-containing complexes. Synthetic analogues of the natural Mycoplasma lipopeptides were synthesized and proved to be equally effective. Both synthetic and natural bacterial lipopeptides were previously shown to activate B-cells, monocytes, neutrophils, and platelets and to act as potent immunoadjuvants in vivo and in vitro. We have investigated the properties of one the well-known synthetic lipopeptides, R,R-Pam2Cys-SKKKK, designated CBLB601, as a radiation countermeasure in mouse model system. This drug was the first in the CBLB600 series of radioprotectors developed by optimization of the peptide portion of lipopeptides. CBLB600 drugs show outstanding radioprotective efficacy rescuing 100% of mice from mortality elicited by 10 Gy total body irradiation (LD100/30) when injected 24 hrs prior to irradiation. DMF30 for CBLB600s obtained in optimal administration conditions is ~1.6. Significant radioprotection was also observed when the drugs were administered at any time between 48 hrs and 30 minutes prior to irradiation. At irradiation dose of LD90/30, CBLB600s administered between 15 minutes and 9 hrs after irradiation also mitigate radiation injury rescuing up to 70% of mice. Radioprotection by CBLB600s is TLR2-dependent since the drugs are ineffective in TLR2 KO mice. Mice protected by CBLB600s survived for at least 9 months after lethal irradiation showing no signs of hematopoietic failure. This result was indicative for effective rescue of long-term repopulating hematopoietic stem cells (HSC) by CBLB600s and prompted us to test the influence of the drugs on HSC. Our data indicate that in vivo administration of CBLB600s to mice and monkeys not only results in increased amount of almost all HSC populations in bone marrow 24 h after injection but also in a robust accumulation of HSC in blood peaking at 72 hours after the drug administration. HSC stimulating and mobilization activity of CBLB600s may be attributed to increased production of G-CSF as detected in our assays and/or with direct stimulation of HSC via triggering TLR2 receptor (Nagai et al, 2006, Immunity 24, 801-12)