New Strategies for the Prevention of Radiation Injury: Possible Implications for Countering Radiation Hazards of Long-term Space Travel

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Radiation injury / Radioprotectors / Space travel / Prophylaxis / Therapy

New strategies for the prevention of radiation injuries are currently being explored with the ultimate aim of developing globally radioprotective, nontoxic pharmacologics. The prophylactic treatments under review encompass such diverse pharmacologic classes as novel immunomodulators, nutritional antioxidants, and cytokines. An immunomodulator that shows promise is 5-androstenediol (AED), a well-tolerated, long-acting androstene steroid with broad-spectrum radioprotective attributes that include not only protection against acute tissue injury, but also reduced susceptibility to infectious agents, as well as reduced rates of neoplastic transformation. Other potentially useful radioprotectants currently under study include the nutraceutical vitamin E and analogs, a chemically-engineered cytokine, interleukin-1β, and a sustained-release formulation of an aminothiol, amifostine. Results suggest that a new paradigm is evolving for the prophylaxes of radiation injuries, based on use of newly identified, nontoxic, broad-spectrum prophylactic agents whose protective action may be leveraged by subsequent postexposure use of cytokines with organ-specific reparative functions.

INTRODUCTION

The three of the most significant health risks associated with space travel, especially those in deep space for extended periods, are (1) ionizing radiation, (2) loss of bone density, and (3) behavioral adaptation1. Health risks associated with radiation exposure are sizable, however those risk estimates have significant degrees of uncertainty. Of most concern are the long-term health effects of chronic exposures to low fluences of extremely high-energy, high-atomic weight particles (HZEs) that comprise, along with high-energy protons, a small but significant component of the galactic cosmic rays (GCRs). Near-term health hazards associated with acute radiation exposures are of much lower probability, but still are of considerable concern due to the immediacy of the exposure effect on in-flight wellness and function2. Estimated radiation exposures during extended space travel are not trivial, especially for extended travel in deep space; e.g., dose estimates for the Mars Mission are of the order of several Sieverts3. The ways and means to minimize these exposures and to protect the astronaut against these health threats need to be developed and implemented.

A research strategy has been adopted based on the
assumptions that: (1) a radioprotective prophylactic agent, however modest in terms of efficacy, can provide a significant protection if leveraged by effective post-exposure recombinant cytokine/growth factor therapies; and (2) considering the current void in radioprotective medicinals suitable for field use, even modestly radioprotective agents should be considered as viable candidates so long as they can be safely administered, are well-tolerated, and produce no adverse physiological and/or performance effects. By adopting this strategy, a number of surprising, promising candidate agents have presented, including nutraceuticals, chemically engineered recombinants, and more conventional pharmacologics. Several of these agents that might prove useful in space medicine, are described in this paper relative to their radioprotective, toxicological, and pharmacological attributes.

MATERIALS AND METHODS

**Animals**

Male C3H/HEN and CD2F1 mice were obtained from the National Cancer Institute (Frederick, MD) and the Jackson Laboratory, Bar Harbor, ME, respectively. All animal-based protocols described were done in accordance with the Guide for the Care and Use of Laboratory Animals and with authorization of the Institutional Animal Care and Use Committee.

**Irradiation**

Mice were bilaterally exposed to $^{60}$Co gamma rays to doses ranging from 0.25–16 Gy and at dose-rates of either 0.4 or 0.6 Gy min$^{-1}$. Details of the exposure and dosimetry procedures are reported elsewhere.

**Drug administrations**

Amifostine, (Ami) 25–745 mg/kg WR-2721 was dissolved in sterile buffered saline (PBS) and injected subcutaneously (SC) in small volumes (0.1–0.2 ml) approximately ~30 minutes prior to irradiation or sham irradiation. WR-2721 was obtained from the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. Slow-release, biodegradable pellets containing 6.25 mg amifostine (Ami-sr) were implanted into the hind legs of mice, 30 minutes to 4 hr prior to acute, lethal irradiation (8–16 Gy). 5-androstenediol, (5-AED) 10–320 mg/kg dissolved in polyethylene glycol-400 vehicle, was injected (0.1 ml) SC into the nape of the neck ~1 day prior to lethal irradiation. Cytokine IL-1β analog, a chemically engineered radioprotective nanopeptide domain (IL-1β-rd) with palmitoyl ester linkage was administered at 80 ug/kg by SC injection (nape of neck) using a PBS vehicle.

**Survival assays**

The efficacy of specific pharmacologics to enhance whole-body radioresistance to total body irradiation (TBI) was assessed by two variants of a conventional 30-day survival-based assay, i.e., a simple 2–3 dose assay or an extended 5-dose assay in order to determine a dose-response factor (DRF). These survival assays have been reported in detail elsewhere.

**Clinical assays**

Complete blood counts (CBC’s) and blood differentials were determined using an Advia automated hematology instrument (Bayer). Complete clinical chemistry panels (19 analytes) were developed on the blood sera of mice using a Vitros 250 instrument (Johnson & Johnson, Inc).

**Experimental hematology assays**

Bone Marrow Cellularity and Cytomorphology. Marrow samples were collected from surgically excised and flushed femurs of euthanized mice. Nucleated cells were counted using a Coulter Z2 cell and particle counter, and used to estimate cellularity of femoral marrow. Impression smears of the extruded marrow were stained with Wrights-Giemsa and examined by light microscopy for cytological evaluations. Progenitor assays. Multipotential cKit$^+$ lin$^-$ progenitors in blood and marrow specimens were quantitated by flow cytometry using a method originally described by Orlic et al. and later modified. Multipotential Granulocyte-Erythroid-Macrophage-Megakaryocyte Colony Forming Cell Assay (GEMM-CFU assay) were assayed using a methylcellulose-based colony assay described by Cortdy. Bipotential Granulocyte-Macrophage Colony Forming Cells (GM-CFC) were assayed using a conventional single layer agar assay as described previously.

**Statistical analysis**

Data from the survival-based assays were compared using the generalized Savage (Mantel-Cox) procedure.
DRFs were estimated from the probit analysis performed on the resulting mortality data. Hematology data from drug and vehicle-treated animals were compared and evaluated using commercially available statistical software (SigmaStat 5.0). For tests of statistical significance, the Student’s T test was applied between test and control groups. Significant differences between groups were defined by P values less than 0.05.

RESULTS

Four classes of radioprotectants show promise as potential safe and effective prophylactic candidates for field use. These agents under test include: (1) the androstene steroid, 5-AED\(^8\), (2) Vit E and structural analogs\(^10\), (3) the cytokine IL-1\(\beta\) and its radioprotective nanoparticle domain (IL-1\(\beta\)-rd)\(^11\); and (4) the aminothiol, Ami (WR-2721) that is administered either at low doses or through sustained-release formulations (Ami-sr) in order to control toxic side-effects\(^7\). The major attributes of these protectants are listed in Table 1. All of these agents appear moderately radioprotective as evidenced by either a significant survival enhancement at single radiation dose levels (e.g., IL-1\(\beta\)-rd), or by the estimated “dose-reduction-factors” (DRFs) of ~1.2–1.3 (e.g., 5-AED, Vit E, Ami-sr). Figure 1 illustrates the degree to which these agents, e.g., Vit E, can significantly enhance 30-day survival of prophylaxed mice prior to receiving graded doses of supra-lethal TBI. The top three agents on the list (Table 1), 5-AED, IL-1\(\beta\)-rd, and Vit E all appear to be well-tolerated and non-toxic when administered doses in excess of radioprotective doses. By comparison, low doses of Ami (100 mg/kg or below) given as a bolus, also appears to be non-toxic and well tolerated by mice, but at higher doses, drug toxicity becomes quite apparent, i.e., behavioral and locomotor dysfunctions become evident\(^7\). In this regard, sustained-release formulations of Ami (Ami-sr) delivered as implanted biodegradable pellets can delay, and to some extend minimize drug toxicity, but cannot eliminate toxicity entirely\(^7\).

In comparing other attributes of these protectants, marked differences are noted: first, in terms of effective time-windows for prophylaxis, Ami has very narrow time window, i.e., in the range of ~15–45 minutes, whereas Ami-sr has an extended time window for effective prophylaxis of ~2 hours. By contrast, 5-AED, Vit E, and IL-1\(\beta\)-rd, all have extended time windows in the range of ~24 hours. Second, in terms of routes of drug administration, 5-AED is effective when delivered either by injection or orally. By contrast, Ami, Ami-sr, Vit E, and IL-1\(\beta\)-rd all appear to be effective only when delivered by injection or by implantation (as in the case of Ami-sr). It should be noted however that analogs of Vit E that are more hydrophilic in nature, can be delivered orally and can afford moderate levels of radioprotection (unpublished preliminary data)\(^16,17\). Third, the pharmacokinetic profiles of Vit E and 5-AED (5-AED pharmacokinetic data derived from canine experiments; data not shown) appear distinct from the pharmacokinetic profiles of both Ami and Ami-sr, in that secondary blood plasma peaks are noted one day or several days following Vit E and 5-AED drug administrations, respectively.

DISCUSSION

Due to the unique radiation environment in deep space, astronauts will be exposed to ionizing radiation of sufficient intensity and for sufficient periods to be deemed at a health risk\(^1\). Few people would argue with this statement. However, what is arguable is the level of the risk and how best to minimize it.

A second unarguable statement is that radiological risk can be effectively managed by controlling the extent of radiation exposure. A corollary to this statement is that radiological risk can be managed by controlling the level of bio-
logical damage imparted by the exposure. How does one develop and install these radiological controls? This no doubt will be a difficult task as the estimated cumulative doses to astronauts during the course of extended missions (e.g., 1000 day Mars mission) are quite large and perceived to be quite hazardous in terms of relative risks. In terms of trying to limit the extent of biological damage, safe and effective medical countermeasures will be needed. Progress into the medical area lags behind other initiatives in safe-effective medical countermeasures will be needed. Progress trying to limit the extent of biological damage, safe and overall safety profiles, and to a degree efficacy profiles, are and specificity of protection afforded, nevertheless their overall safety profiles, and to a degree efficacy profiles, are sufficient to warrant serious consideration as medical countermeasures for space-associated radiation hazards. Take for example the listed prophylactic agents (Table 1): several fall into the “nutraceutical” category and are already taken routinely by very large numbers of individuals around the world without any apparent undue health effects. It would seem reasonable, therefore, to suggest that an agent such as a hydrophilic formulation of Vit E could be taken by astronauts on a periodic basis during space flight in order to provide not only needed nutritional supplementation, but also a degree of radioprotection. Even the other two protectants listed, 5-AED and Ami-sr, might be considered after additional safety and toxicity evaluations.

In addition to our work, other investigators (several participants of ISWWRR-2 workshop in Nara, Japan, March 11–15, 2002) have examined a number of different nutritional- and pharmacological-based agents for their radioprotective attributes using a variety of in vitro and in vivo test systems and have made comparable observations and have arrived at similar conclusions concerning the utility of these “dietary” and “over-the-counter” pharmaceuticals for radio-

Table 1. Characteristics of radioprotective prophylactic agents currently under test

<table>
<thead>
<tr>
<th>Agent</th>
<th>Route</th>
<th>Dosage</th>
<th>Efficacy</th>
<th>Window</th>
<th>P-Kinetics</th>
<th>Toxicity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-AED</td>
<td>Inject-sc</td>
<td>10-360 mg/kg 1.6 gm/kg</td>
<td>DRF = 1.26 60% Survival - 11 Gy</td>
<td>24 h preexposure 2 h postexposure</td>
<td>nd</td>
<td>Local inject site nd (no apparent toxicity)</td>
<td>8,9</td>
</tr>
<tr>
<td>Vit E</td>
<td>Inject-sc</td>
<td>100-400 U/kg</td>
<td>75% Survival - 10.5 Gy</td>
<td>DRF = 1.23</td>
<td>20–24 hr pre-exposure</td>
<td>4 &amp; 24 h peaks; w. rad 24 h peak</td>
<td>nd (no apparent toxicity)</td>
</tr>
<tr>
<td>IL-1β-rd</td>
<td>Inject-sc</td>
<td>80 µg/kg</td>
<td>40% Survival - 8.5 Gy</td>
<td>DRF = nd</td>
<td>24 hr pre-exposure</td>
<td>nd</td>
<td>(no apparent toxicity)</td>
</tr>
<tr>
<td>Ami-sr</td>
<td>Inject-sc</td>
<td>100 mg/kg</td>
<td>DRF = ~1.3</td>
<td>0.5 h pre-exposure ~ 2 h</td>
<td>nd (no apparent toxicity)</td>
<td>(no apparent toxicity)</td>
<td>Delayed locomotor effects</td>
</tr>
<tr>
<td>Ami-sr</td>
<td>Implant-sc</td>
<td>100 mg/kg/h</td>
<td>DRF = ~2.0</td>
<td>15–30 min peak</td>
<td>broad ~ 1h peak</td>
<td>(no apparent toxicity)</td>
<td>Delayed locomotor effects</td>
</tr>
</tbody>
</table>

1 Agents. 5-AED = 5-androstenediol; Vit E = vitamin E (alpha tocopherol); IL-1β-rd = interleukin one beta-radioprotective domain; Ami-sr = amifostine- slow release.
2 Route. Inject. sc = drug delivered by subcutaneous injection; oral gavage = drug delivered orally by a feeding tube; implant-sc = slow release Ami pellets implanted subcutaneously with a trocar.
3 Dosage. Amount of drug injected in either milligrams (mg) or international units (U) per kilograms (kg) of body weight per day (d).
4 Efficacy. DRF = dose reduction factor, as estimated by the ratios of LD50/30 values for the drug-treated versus vehicle-treated animals. Percentages of surviving animals within groups given selected radiation doses of either 8.5, 10.5, 11 Gy.
5 Window. Pre-irradiation periods (in hours) in which the applied prophylactic agents were shown to be most effective in terms of enhancing survival (at 30 days).
6 P-kinetics. Pharmacokinetics of administered drugs relative to the times in which peak blood levels were noted. Notation of “nd” indicates that the determinations have not yet been made.
7 Toxicity. Specific sites of toxicity were noted (e.g., injection site with AED). “nd” notation indicates that full complement of toxicity tests were not preformed. The notation of “no apparent toxicity” indicates that the treated animals exhibited no obvious clinical toxicity following drug administration. “Delayed locomotor effects” notation indicates that delayed behavioral responses were detected in the Ami-sr-treated animals.
8 References. Citations of related work previously presented/published by either the authors, or by others.
protective purposes in both space travel and in earth-bound settings. Several of those agents that have proven to be efficacious from a radioprotective standpoint include: dietary citrus juices in preventing HZE (iron 56)-mediated cognitive behavioral changes in experimental rats (Shukitt-Hale B., et al. ISWWRR-2, 2002); vitamin C treatments in quenching free radical mediated mutations within HZEs (carbon) (Ueno, A.M., et al. ISWWRR-2, 2002); dietary retinyl acetate in blocking radiation-induced skin cancers in rats exposed to various qualities of ionizing irradiation, including heavy iron particles (Burns, F.J. et al. ISWWRR-2, 2002); dietary retinyl acetate in blocking radiation-induced skin cancers in rats exposed to various qualities of ionizing irradiation, including heavy iron particles (Burns, F.J. et al. ISWWRR-2, 2002); dietary minerals, e.g., sodium tungstate19). In addition, Kagiya and colleagues 16,20) are working on Vit E analogs in much the same manner as we are here at AFRRI. One particular analog under investigation appears particularly promising, namely [2(alpha-D-glycopyranosyl) meth- yl- 2,5,7,8-tetramethylchroman-6-ol] or alpha-TMG for short. Due to alpha-TMG’s water solubility, it can be delivered either orally or by injection, and can provide modest levels of survival protection; i.e, DRFs of 1.08 and 1.12 for respective administration by oral and by injection have been estimated. Improved safety and efficacy profiles might be possible by chemically engineering such radioprotective agents for greater specificity and potency (e.g, engineered active domains of anti-apoptotic molecules).21)

We fully recognize and acknowledge that these prophylactic agents might not be sufficiently potent by themselves in providing total protection against radiation injury that stems from various exposure scenarios (e.g., chronic exposures to low fluence HZE), but even if partial protection can be provided it would be better than having no protection, especially if the protection can be delivered simply and safely as an integrated part of the astronaut’s normal diet. This concept is certainly not novel, but has been stated in various ways by a number of investigators and scientific review bodies, over the last decade or so.

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