

# BIOLOGICAL COUNTERMEASURES IN SPACE RADIATION HEALTH

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## ABSTRACT

Exposure to the types of ionizing radiation encountered during space travel may cause a number of health-related problems, but the primary concern is related to the increased risk of cancer induction in astronauts. The major types of radiation considered to be of importance during space travel are protons and particles of high atomic number and high energy (HZE particles). It is now clear that biological countermeasures can be used to prevent or reduce the levels of biological consequences resulting from exposure to protons or HZE particles, including the induction of cancer, immunosuppression and neurological defects caused by these types of ionizing radiation. Research related to the dietary additions of agents to minimize the risks of developing health-related problems which can result from exposure to space radiations is reviewed.

**Key words:** space radiation health, countermeasures in space, antioxidant, carcinogenesis, immunology, neurological effects

## INTRODUCTION

As noted in previous articles in this series, the types of radiation that are of concern in space flight can be categorized into constant and stochastic sources. The former consist of trapped particles in Earth's radiation belts, solar protons and helium ions beyond Earth's magnetosphere and galactic cosmic ray HZE particles. The latter consist of high proton fluxes from solar proton events. Likewise the biological risks are also categorized as deterministic and stochastic risks. The former consists of the acute somatic effects of solar proton storms due primarily to cell killing. The latter consist of cancer induction and neurological effects. This situation produces the following enigma: the stochastic source of radiation causes deterministic effects; the constant radiation source causes the stochastic effects. A crew member will thus never know, *a priori*, whether he or she will be one of those who get cancer or one of those who will be subjected to a high-dose (> 1 Gy) solar proton exposure, or both. Research over the past several years has been conducted to discover biological countermeasures to these three risks: carcinogenesis by HZE particles, immune system effects due to high doses plus life in low gravity, and subtle neurological and behavioral effects that might jeopardize a deep-space mission. The general findings to date indicate that dietary antioxidants can constitute a line of defense against all of these radiation risks. In addition, cytokine, hormone and dietary nucleoside therapies may also be found to be useful.

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## CARCINOGENESIS COUNTERMEASURES

The induction of cancer is a highly modifiable phenomenon. At this point, numerous agents have been shown to either enhance or suppress the carcinogenic process induced by ionizing radiation in both *in vivo* and *in vitro* carcinogenesis systems. It was previously believed that the induction of cancer by HZE particles, representing a form of high LET (linear energy transfer) radiation, might not be a modifiable phenomenon. Recent research has indicated that carcinogenesis induced by HZE particles can be suppressed by retinyl acetate (Burns *et al.*, 2001) and tamoxifen (Drs. John Dicello, David Huso and other colleagues, unpublished data). Thus, "proof of principle" has been achieved in this area of research. Unfortunately, significant toxicities and adverse side effects have been associated with retinoids, such as retinyl acetate, and with tamoxifen in human trials which have been performed. The challenge to researchers in this field now is to find agents that can prevent cancer induced by exposure to HZE particles and other types of space radiation in multiple organ or tissue sites without significant toxicities or adverse side effects.

Many agents that have been classified as dietary supplements are currently being evaluated as cancer preventive agents. It is expected that many of these dietary supplement agents will be able to serve as cancer preventive agents during space travel without having the toxicities or adverse side effects associated with retinoids or tamoxifen. Current research in the Kennedy laboratory is focused on the development of a dietary supplement formulation to protect astronauts from the biological effects expected from exposure to the types of radiation encountered during space travel, with emphasis on the prevention of cancer induced by space radiation.

## PROTEASE INHIBITORS

Certain protease inhibitors are extremely effective at preventing or suppressing radiation induced transformation *in vitro* and carcinogenesis in animal model systems (reviewed in references (Kennedy, 1998a,b). One protease inhibitor, the soybean-derived Bowman-Birk inhibitor (BBI), has been particularly effective in suppressing the carcinogenic process; BBI has been developed specifically as an agent to prevent radiation-induced cancer. BBI has been extensively studied, both as purified BBI (PBBI) and as an extract of soybeans enriched in BBI, called BBI Concentrate (BBIC). BBI appears to be a universal cancer preventive agent. PBBI and/or BBIC have been shown to suppress carcinogenesis: 1) in three different species (mice, rats and hamsters), 2) in several organ systems and tissue types (colon, liver, lung, esophagus, cheek pouch (oral epithelium) and in cells of hematopoietic origin), and 3) in cells of both epithelial and connective tissue origin. When given to animals by several different routes of

administration (including the diet), PBBI and/or BBIC can suppress carcinogenesis leading to several different types of cancer (e.g., squamous cell carcinomas, adenocarcinomas, angio-sarcomas, etc.) induced by a wide variety of chemical and physical carcinogens. BBI was originally identified as an anticarcinogenic agent by its ability to prevent radiation-induced transformation *in vitro*. Both BBI and BBIC prevent the radiation induced malignant transformation of cells in culture, and cancer in animals, without toxicity or adverse side effects (Kennedy, 1998b). As BBI has been shown to prevent carcinogenesis in a variety of *in vivo* and *in vitro* systems, it is expected that it will also suppress carcinogenesis induced by HZE particles and protons. It has been observed that BBI inhibits the initiation, promotion and progression stages of carcinogenesis in cells and animals. BBI has also been shown to be a radioprotective agent (Kennedy *et al.*, 1996; Dittman *et al.*, 1995).

### ANTIOXIDANTS AND AGENTS AFFECTING FREE-RADICAL LEVELS IN IRRADIATED TISSUES

Free radicals and active oxygen species are generated immediately in irradiated tissue. The availability of radical scavenging and competing compounds during exposure to ionizing radiation is known to chemically ameliorate the immediate molecular damage and hence cellular responses. As the damaged cell proceeds through further steps toward malignancy long after irradiation, its health continues to be affected by oxidative stress (Sies, 1985). Therefore, the first and second lines of defense both consist of the maintenance of a highly reductive environment within the cell. The following paragraphs summarize various means of ameliorating carcinogenesis in general and radiation carcinogenesis in space in particular.

Well-known antioxidant agents include vitamins C (ascorbic acid) and E (d- $\alpha$ -tocopherol), alpha-lipoic acid, niacin (vitamin B3), thiamin (vitamin B1), folic acid, glutathione (GSH), N-acetylcysteine, Co-enzyme Q10 and selenium. There is a large amount of information available on these dietary supplements; the Healthworld (a part of the World Health Organization) Internet Site descriptions of these agents are recommended reading. These agents will be described only briefly here. Vitamin C is a water-soluble antioxidant that protects cells by interacting with hydroxyl radicals to form less toxic ascorbate free radicals, which can be detoxified by enzymes that reduce ascorbate free radicals back to ascorbic acid (Rose, 1990). Vitamin E is a lipophilic agent that protects cell membranes from oxidative damage induced by radiation or other physical or chemical agents (Livrea and Tesoriere, 1998; Wolf *et al.*, 1998). The suppressive effect of vitamin E on radiation-induced carcinogenesis has been demonstrated both in *in vitro* (Radner and Kennedy, 1986; Kennedy, 1990) and in animal studies (Prasad and Edwards-Prasad, 1992). For example, by feeding vitamin E Trickler and Shklar

(1987) demonstrated a reduction to zero tumors in 20 animals compared to 49 tumors per 20 animals in the hamster cheek-pouch model using dimethylbenzanthracene as the carcinogen. The dietary supplementation of vitamins C and E is considered to be important in protecting against human diseases associated with free radical damage to cellular DNA, lipids and proteins (Jacob and Burri, 1996). Increased vitamin C and E intake has been associated with a reduced risk of cancer development (Colacchio and Memoli, 1986; Cook and McNamara, 1980). Lipoic acid, a B vitamin, is a potent antioxidant which is both lipid and water-soluble, and reacts chemically with hydroxy radicals, singlet oxygen, and peroxy and hypochlorous radicals. Lipoic acid had been called a "universal antioxidant" (Packer *et al.*, 1995). It is an excellent radical scavenger in both the oxidized and reduced form, and is known to regenerate other antioxidants from their inactive forms. It also protects cell membranes by interacting with vitamin C and glutathione, which may in turn recycle vitamin E (Packer *et al.*, 1995, 1997). Treatment with lipoic acid has been shown to protect cells in a number of oxidative stress models, including radiation-induced oxidative stress (Biewenga *et al.*, 1997; Marangon *et al.*, 1999). Treatment with lipoic acid together with vitamins C and E has been shown to protect against lens damage caused by low level radiation in astronauts (Bantsev *et al.*, 1997). Niacin (also known as vitamin B-3) is a water-soluble vitamin that has been shown to prevent cancer induced by ultraviolet light (Gensler *et al.*, 1999) and chemical carcinogens (van Rensburg *et al.*, 1986). Thiamin (vitamin B-1) is a co-factor for enzymes required to bridge aerobic and anaerobic metabolism in cells, and thiamin deprivation often results in systemic oxidative stress linked to degenerative diseases (Frederikse *et al.*, 1999). In a few studies, a high level of thiamin intake has been associated with a reduced risk of cancers in the colon (Slattery *et al.*, 1997), prostate (Kaul *et al.*, 1987) and esophagus (Ziegler *et al.*, 1981). Epidemiologic data and human and animal studies strongly suggest that increased folate status reduces the risk of developing cancer (Newburne and Lochniscar, 1990; Choi and Mason, 2000). Glutathione is a tripeptide small molecular weight thiol that is a versatile protector against radiation induced oxidative damage (Bump and Brown, 1990). N-acetylcysteine is also a small molecular weight thiol that serves as a precursor to intracellular cysteine and glutathione. Both N-acetylcysteine and glutathione have been shown to be effective in reducing the development of cancer and are considered to be promising cancer preventive agents (van Zandwijk, 1995; Slaga, 1995). Coenzyme Q10 is an essential electron and proton carrier that functions in the production of biochemical energy in aerobic organisms and is produced in all mammalian tissues. Coenzyme Q10 also has antioxidant and membrane-stabilizing properties that protect cells from oxidative damage at physiological concentrations (Frei *et al.*, 1990). Treatment with coenzyme Q10 was shown to result in partial or complete regression of breast cancer in some patients (Lockwood

*et al.*, 1994, 1995). Selenium is a trace element that is essential in the enzyme activity of thioredoxin reductase and glutathione peroxidase activity. It functions with vitamin E to protect cell and organelle membranes from oxidative damage, to facilitate the union between oxygen and hydrogen at the end of the metabolic chain, and to transfer ions across cell membranes (Frost and Lish, 1975). Selenium has been shown to be a promising cancer preventive agent in several studies (Reddy *et al.*, 1997; Nelson *et al.*, 1999; Khuri and Lippman, 2000), including several large-scale human intervention trials (Clark *et al.*, 1996; Li *et al.*, 1993). For example, as shown in Table 1, Medina *et al.* (1983) demonstrated a three-fold reduction in dimethylbenzanthracene-induced mammary tumors in

**Table 1. Effect of dietary selenium (as sodium selenite) on dimethylbenzanthracene-induced tumors in female BD2F<sub>1</sub> mice (Medina *et al.*, 1983).**

| Dietary Selenium (ppm) | Mammary Tumors/Mice | Percent Mice With Tumors | Months to 25% Tumor Incidence |
|------------------------|---------------------|--------------------------|-------------------------------|
| 0.20                   | 18 / 32             | 56                       | 7.0                           |
| 2.00                   | 5 / 32              | 16                       | > 9.0                         |

female mice. Most of the cancer preventive agents discussed here are antioxidants that reduce oxidative stress in the cells. BBI may also function as an antioxidant as it reduces lipid peroxidation in UV-irradiated 3T3 mouse fibroblast cells (Baturay and Rogue, 1991) and suppresses the production of superoxide anion radicals in differentiated HL-60 human lymphocytes (Ware *et al.*, 1999) and in stimulated polymorphonuclear leukocytes (Frenkel *et al.*, 1987). BBI could therefore also serve as an immune-system protector (see below).

If uncontrolled, oxidative stress may lead to the ultimate consequences of radiation-induced biological damage, such as cancer; thus, the agents recommended as dietary supplements are likely to prevent biological damage induced by the various types of ionizing radiation and other agents encountered during space travel (Stein *et al.*, 2000). Most of these agents have long histories of safe use in people or are more recently studied agents which have been shown to be non-toxic and safe for use in people over a particular range of doses specified for their Investigational New Drug status with the FDA. Thus, it is expected that effective levels of all of these agents (i.e., levels that can serve as radioprotective agents) can be administered to humans without producing toxicity or adverse side effects. However, the relative amounts of each agent and the most effective agents or combinations of agents for protection against radiation induced biologic effects are not known at this time.

It is clear from animal studies that vitamins and minerals, and deficiencies of these agents, can play major roles in carcinogenesis. It has been shown that adequate dietary levels of several different vitamins and minerals reduce the risk of cancer (Newberne and Rogers, 1985; Nelson, 1987). Human intervention studies have shown

that vitamin and mineral supplements can have a major preventive effect on carcinogenesis in populations that are nutritionally deficient for these dietary supplements (Li *et al.*, 1993; Blot *et al.*, 1993). With the oxidative stress produced by space travel, it is conceivable that astronauts may deplete their vitamins and mineral stores more rapidly than other individuals and may need considerably higher amounts of antioxidant vitamins and minerals than the established Recommended Dietary Allowances (RDAs). It has been pointed out by Bruce Ames that micronutrient deficiencies are a major cause of DNA damage, and deficiencies in the vitamins B<sub>12</sub>, B<sub>6</sub>, C, E, folate, niacin, iron and zinc mimic radiation in damaging DNA by causing single and double strand DNA breaks, oxidative lesions or both (Ames, 1999). Thus, supplementation with antioxidant vitamins is likely to reduce the radiation-induced damage associated with space travel.

The succinate form of vitamin E has the ability to prevent radiation-induced cell transformation *in vitro* while other forms of vitamin E do not have this ability (Radner and Kennedy, 1986). Similarly, vitamin E succinate has been the most active form of vitamin E in many other types of cancer prevention experiments in the laboratory (Prasad and Edwards-Prasad, 1992). The form of selenium appears to be extremely important for cancer preventive effects. In animals, organoselenium forms work better than inorganic forms of selenium (El-Bayoumy *et al.*, 1995), although selenomethionine has been shown to be ineffective for colon cancer prevention (Reddy *et al.*, 2000).

## THE SURROGATE ENDPOINT BIOMARKERS CONCEPT

Many different agents have been shown to prevent carcinogenesis in *in vitro* systems, including many of the agents proposed for use as countermeasures in this document, and promising agents from *in vitro* studies have gone on to studies in animals and human populations in the past. Every new compound or intervention protocol obviously cannot be subjected to animal or human interventional studies. A current requirement for a chemopreventive agent or agent combination to go on to large-scale intervention trials in their evaluation as a human cancer chemopreventive agent is a clear effect of the potential agent on surrogate endpoint biomarkers (SEBs) in the carcinogenic process (Kelloff *et al.*, 2000). An example of the use of SEBs in a cancer prevention trial utilizing BBI as the cancer preventive agent is found in the study by Wan *et al.* (1999). The cellular bio-reduction capacity is an example of a useful SEB because we believe that the cellular bio-reduction capacity constitutes the first line of cellular defense, which scavenges radiation-generated free radicals and reactive oxygen species or converts them into non-toxic molecules. When the cellular bio-reduction capacity is overwhelmed, free radicals and reactive oxygen species will accumulate to cause oxidative stress and damage cellular macromolecules (DNA, lipids and proteins). Oxidative stress levels can be determined by the

dichlorofluorescein (DCF) fluorescence assay in cells and in animals by the protein carbonyl measurement. In the DCF fluorescence assay the intensity of fluorescence is proportional to the concentration of hydrogen peroxide, and the protein carbonyl measurement reflects the amount of protein oxidative damage. We hypothesize that the maximal bio-reduction potential of cells is directly related to their ability to survive oxidative stress produced by ionizing radiation. We also believe that optimal protection from radiation induced oxidative damage occurs when cellular protein and non-protein thiols (GSH) are high, selenium induced peroxidases are at maximum activity, and the overall bioreduction state of the cell is maximal.

### IMMUNE SYSTEM COUNTERMEASURES

The immune system is affected by both radiation and space flight conditions. A solar proton storm dose of 1.0 Gy delivered in a 3-day period could be lethal to crew members whose immune systems have been compromised (Parsons and Townsend, 2000; Todd *et al.*, 1999). Most of the responses of the human immune system are blunted by exposure to the conditions of space flight, including both humoral and cell-mediated immunity, redistribution of T-cell subgroups, delayed hypersensitivity, post-mission *in vitro* blastogenic response and interleukin production, and macrophage differentiation (Taylor, 1993; Konstantinova *et al.*, 1991, 1993). The corresponding events occur in test animals (Lesnyak *et al.*, 1993), and a fully analogous set of responses is seen during antiorthostatic hind-limb suspension of test animals on the ground (Chapes *et al.*, 1993).

The general cause of the severe depression of the immune system by ionizing radiation is stem cell depletion. For example, stem cell depletion is a common consequence of certain cancer treatments involving radiation or cytotoxic agents. Some of the following features of the immune response have been reviewed in the context of radiation sensitivity of space travelers (Todd *et al.*, 1999): IL-4 based CD4+ activity is radiation sensitive, apoptotic death occurs in TcR- $\gamma/\delta$  T lymphocytes and NK cells lacking p53, anti-idiotypic antibody presentation is radiation sensitive, and T-lymphocyte sensitivity is clone dependent. Cytokines (interleukins) have potential as radioprotective agents; for example, IL-2 rescues T-cells when Bcl-2 is induced, cytokines differentially protect immune-system cells against apoptosis, IL-12 protects bone marrow but sensitizes the intestine. Cytokine treatment for immunosuppression caused by space flight conditions other than radiation has not been adequately tested, and Pecaut (1999), for example, found no statistically significant evidence for an effect of IGF-1 on the space-flight-induced redistribution of three cell types, T4, T8 and granulocytes in the rat spleen.

As an alternative, dietary nucleic acid precursors have been proposed as protection for space travelers. Mononucleotides available in cells via scavenger pathways are incorporated into DNA during post-irradiation DNA repair. Years of study using radioisotope and halopyrimidine labeling methods have depended

on this fact. Generally the directing of exogenous mononucleotides into cell DNA is accomplished by the supplying of exogenous nucleosides in the culture medium or by injections into intact organisms. It has thus been postulated that immune functions requiring the synthesis of nucleic acids (progenitor cell multiplication) might benefit from enhanced levels of exogenous nucleosides. The numerous ways in which exogenous nucleosides might aid the health of the challenged cell or organism have been systematized by Kulkarni *et al.* (2002), who tested immune function (popliteal lymph node proliferative response, cytokine production, splenocyte proliferative response) in mice. All of these functions were suppressed by hind-limb suspension, and all functions were normal or above when suspended animals were fed chow diets containing 0.06% uracil, and some functions (proliferative responses) benefited from the addition of 0.25% purified yeast RNA into the standard chow diet. The pleiotropic effects that have been attributed to nucleotide-precursor supplemented diets in laboratory animals include decreased infection rates, increased cytokine production, increased maturation of stem cells to T lymphocytes, decreased melanoma metastasis, improved wound healing, and increased blastogenesis in mixed leukocyte culture (Hales *et al.*, 2002; Kulkarni *et al.*, 2002).

### NERVOUS SYSTEM COUNTERMEASURES

A wide variety of morphological and physiological changes in neural systems have been reported following heavy-ion irradiation, usually at rather high doses, typically exceeding 1 Gy (Joseph *et al.*, 1998). Recalling that more than half of all cells are hit by at least one cosmic ray in a 2–3-year deep-space mission, it is necessary to ask if the neurological functioning of crew members will be impaired to such an extent as to jeopardize a mission and/or the lives of the crew members. Laboratory animal studies are now underway to determine, via quantitative neurochemistry and behavior analysis, the nature and level of effects on nervous function (Rabin *et al.*, 2000) since functional effects constitute end-points of relevance to mission performance while morphological effects may or may not relate to critical functions.

The following tests have been performed on rats exposed to graded doses of gamma rays, protons and  $^{56}\text{Fe}$  ions: (1) potassium-ion stimulated dopamine release, (2) operant responding (bar press), (3) upper-body strength – reacting by hanging using the front paws to avert a fall, (4) learning in a water maze, and (5) amphetamine-induced conditioned taste aversion (CTA) in which water containing sugar is avoided after the administration of amphetamine, a dopamine agonist, following a drink of sugar solution (Rabin *et al.*, 2000). There was a decrement in all of the tested functions in animals whose heads were exposed to  $^{56}\text{Fe}$  ions, and the threshold dose for detectable performance decrements was about 0.1 Gy. To date, no apparent recovery of functions has been reported. The operant response decrement occurred 11 months after

exposure. Histopathology showed evidence of reduced neurogenesis in 28 days. All of these effects caused by  $^{56}\text{Fe}$  ions were not observed after exposure to protons or  $\gamma$  rays.

After behavioral responses were characterized, countermeasures to the behavioral effects of HZE particles were sought in the form of dietary anti-oxidants. Since plant anthocyanins are thought to have many times the antioxidant power of the antioxidant vitamins E and C, antioxidant diets were compounded by blending 2% blueberry extract or 1% strawberry extract into countermeasure diets and feeding control animals standard diets. Thus the effects of diet on the  $^{56}\text{Fe}$  particle-induced disruption of dopamine-mediated behavior were studied. Three groups of rats were exposed to a 1.5 Gy dose of  $^{56}\text{Fe}$  ions (AGS at Brookhaven National Laboratory), and it was found that this dose disrupted amphetamine-induced conditioned sucrose taste aversion (CTA), but CTA was not disrupted in sham control rats, and not disrupted in exposed rats on the 2% blueberry or strawberry extract diets (Rabin *et al.*, 2001).

### SUMMARY STATEMENTS

It has already been observed that one of the agents proposed for use, vitamin E, reduces the amount of oxidative damage observed in astronauts (Bantsev *et al.*, 1997). In addition, there are now numerous studies indicating that vitamin supplements can reduce the cancer incidence in various populations (Taylor *et al.*, 1994; Patterson *et al.*, 1997; Biasco and Paganelli, 1999). We expect that a dietary supplement can be developed and recommended that will be considerably more effective than any of the agents or agent combinations studied previously. We recognize, for example, that the drug, WR2721, has been previously considered for use by astronauts for protection against radiation induced biological effects. We did not include this drug in our list of potential supplements because we feel that a supplement can be proposed that will be more effective. In addition, use of the above-described agents will not lead to toxic or adverse side effects such as calcium loss.

### NOMENCLATURE AND GLOSSARY

|       |  |
|-------|--|
| AGS   | Alternating Gradient Synchrotron accelerator                                 |
| BBI   | Bowman-Birk soybean protease Inhibitor                                       |
| CTA   | Conditioned Taste Aversion test  |
| DCF   | Dichlorofluorescein  |
| GCR   | Galactic Cosmic Rays, high energy nuclei from outer space                    |
| GSH   | Glutathione  |
| Gy    | Gray, the SI unit of absorbed ionizing radiation dose (1 J/kg, 100 rad)      |
| HZE   | High-charge, high energy particles, GCR                                      |
| IGF-1 | Insulin-like Growth Factor   |
| IL-2  | Interleukin 2  |
| LET   | Linear Energy Transfer, energy deposited per unit distance in particle track |
| RBE   | Relative Biological Effectiveness  |
| SEB   | Surrogate End-point Biomarker  |

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