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SPACE RADIATION HAZARDS AND STRATEGIES FOR ASTRONAUT/COSMONAUT PROTECTION

ОПАСНОСТЬ КОСМИЧЕСКОЙ РАДИАЦИИ И СТРАТЕГИЯ ЗАЩИТЫ АСТРОНАВТОВ/КОСМОНАВТОВ

РЕФЕРАТ

Обзорно-экспериментальная статья посвящена способам защиты участников космических полетов на основе накопленных фундаментальных данных о механизмах радиационного воздействия и окислительных стрессов.

Ключевые слова: *радиация, аэрокосмическая медицина, опасность окружающей среды, окислительное повреждение, защита*

ABSTRACT

Purpose: 1. Discuss the sources of radiation injury and roles of oxidative stress and radiation toxicity. 2. Define the exposure environment of astronauts and cosmonauts working in space and on future exploration–class missions. 3. Review the development of countermeasures for oxidative stress, radiation toxicity and radiation exposure for workers in extreme environments.

Methods: Multiple placebo-controlled, randomized prospective studies have been conducted which have studied the therapeutic and radioprotection effects of various oral, parenteral and combination countermeasures on the biological consequences and survival rates after acute and chronic radiation exposure.

Results: Discussion: Employing oral chemoprevention formulas, parenterally administered MnSOD-plasmid liposomes, and hyperimmune serum and vaccines directed on radiation-induced toxins, have resulted in reduced lipid peroxidation and DNA damage, as well as increased survival in cell cultures and whole animals receiving acute high-dose radiation exposures. Each of these strategies, alone and in combination, deserve further investigation in the pursuit of effective countermeasures and treatment for occupational exposures which induce oxidative damage.

Key words: *Radiation, Space Medicine, Space Environmental Hazards, Oxidative Damage, Countermeasures*

Обзорная часть

Авторами проведен подробный анализ источников радиационных повреждений, которые могут иметь значение при космических полетах. Большое внимание в статье посвящено разбору собственных более ранних публикаций.

Рассмотрена в том числе роль окислительного стресса и окислительных повреждений при неблагоприятных воздействиях. Отмечается, что астронавты/космонавты при возможных полетах на Луну, Марс или околоземные астероиды должны сталкиваться с риском острого и хронического облучения от тяжелых частиц, солнечных частиц и галактических космических лучей. Космическое излучение имеет свою специфику по сравнению с «чистым» рентгеновским и γ-излучением в плане поглощения энергии и степени ионизации. Однако в радиационной эпидемиологии и в радиобиологии слабо изучены эффекты тяжелых частиц, хотя относительно немногие исследования на животных и культурах клеток, облученных высокоэнергетическими протонами и тяжелыми ионами, продемонстрировали отчетливую индукцию онкогенной трансформации *in vitro* и опухолеобразования *in vivo*. Тяжелые ионы имеют более значительный канцерогенный потенциал, чем, к примеру, γ-излучение в связи с особым спектром индуцируемых повреждений ДНК и с особой трудностью их репарации. В связи с этим, основная цель космической радиобиологии — разработка методов оценки и предотвращения потенциальных радиационно-индуцируемых клеточных повреждений, которые могут приводить к ракам или иным патологиям во время или после длительного космического полета.

В предваряющем экспериментальную часть обзоре авторы подробно разбирают молекулярные механизмы биологических последствий воздействия ионизирующего излучения, среди которых ключевыми являются нерепарированные повреждения ДНК, приводящие к мутациям, апоптозу, клеточному старению, канцерогенезу и гибели. Главная причина таких повреждений — формируемые в результате облучения активные формы кислорода и продукты перекисного окисления липидов.

Изменения на молекулярном и клеточном уровнях способны приводить к нарушению функциональных систем организма и к формированию повреждений и патологий *in vivo*, вплоть до тяжелых форм лучевой болезни. В особенности значительным поражающим эффектом обладают тяжелые ионы и космические частицы. Авторы

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разбирают собственное исследование, в котором обнаружено, что крысы, подвергавшиеся воздействию заряженных частиц с высокой энергией от галактического излучения, совершают больше ошибок в процессе обучения и проявляют когнитивные особенности, характерные для периода старения. Причина заключается в том, что высокоэнергетические частицы подавляют дофаминергическую систему, нарушая обучение, память и локомоторные функции даже после воздействия в дозе 0,1 Гр.

Для человека такие экспериментальные данные, понятно, ограничены, но известно, что в течение периода выброса солнечной энергии снижается работоспособность и скорость выполнения определенных задач.

Все это может быть важным в плане полетов на Луну и Марс, тем более, что Луна лишена атмосферы, а Марс имеет разряженную атмосферу, и, вкупе с отсутствием у них магнитосферы, данные моменты приводят к тому, что фон ионизирующего излучения на указанных планетах значительно интенсивнее по сравнению с полетами на околоземной орбите. Отсюда следует, что защита астронавтов/космонавтов при полетах на Луну и Марс должна включать не только физические способы снижения радиационной экспозиции, но и соответствующие фармакологические средства, а также постполетные медицинские процедуры, снижающие последствия лучевого поражения.

В первую очередь необходимо снизить уровень окислительного стресса, который с неизбежностью будет сопутствовать длительным полетам. Авторы перечисляют окислительные агенты, способные вызвать окислительный стресс в космосе, и, цитируя собственные исследования (анализ биохимических маркеров окислительных повреждений до и после полета) показывают, что астронавты на самом деле испытывали во время полета окислительный стресс. Интересным является тот момент, что причины окислительного стресса дифференцированы для двух различных ситуаций: во время космического полета и уже при исследовании планеты. Анализируются все возможные источники окислительных повреждений во время и после полета, включая физические упражнения.

Рассмотренные положения приводят авторов к выводу о необходимости повышения тем или иным путем антиоксидантного статуса астронавтов/космонавтов с параллельной биодозиметрией с помощью известных маркеров (на повреждения ДНК и аберраций хромосом). Для достижения эффекта защиты авторами предлагается прием препаратов по так называемой «oral formula», в составе специального «коктейля», который, с одной стороны, должен повысить активность защитных систем организма (включая антиоксидантные и иммунные) и, с другой стороны, снизить последствия окислительного стресса.

Названная формула, состав которой приведен в статье, включает около 30-ти различных витаминов и нутриентов, для каждого из которых указаны необходимые дозы. В одном из своих исследований авторы с помощью методов молекулярной биологии провели изучение эффективности некоторых компонентов предлагаемой ими «формулы» в условиях *in vitro* и *in vivo* (на мышах линии C57BL/6NHsd). Последним перед облучением в дозе 9,5 Гр внутривенно вводили плазмиду с генами, кодирующими Mn-супероксиддисмутазу (антиоксидантный фермент). Кроме того, некоторым группам мышам давали «антиоксидантую диету» перед радиационным воздействием. Результатом являлся радиозащитный эффект.

Экспериментальная часть

В качестве объекта опытов на животных *in vivo* использовались мыши, крысы, кролики, овцы, свиньи, собаки и крупный рогатый скот. Животных облучали γ-лучами в дозах вплоть до 10 Гр и изучали систему иммунитета, а также выживаемость. Кроме того, в условиях без облучения исследовали воздействие гипериммунной сыворотки или парентеральной вакцины, выделенных из этих облученных животных. Последнее приводило к признакам, характерным для острой лучевой патологии (высокие дозы «радиотоксинов» даже индуцировали гибель). Специальные же антитела, полученные против «радиотоксинов», снижали указанные неблагоприятные последствия и признаки, характерные для лучевой болезни.

На людях была изучена степень окислительного стресса у пилотов (по параметрам перекисного окисления липидов, повреждениям ДНК и суммарной антиоксидантной активности). В случае использования контингентом защитной формулы показатель перекисного окисления липидов был значительно ниже по сравнению с группой плацебо.

Общее заключение

В обзорной части статьи рассмотрены: 1) источники окислительного стресса при космических полетах; 2) сложность радиационного воздействия вдали от геомагнитосферы Земли; 3) некоторые потенциально могущие быть успешными направления исследования для предотвращения и снижения последствий профессионального воздействия окислительного стресса, в особенности острого и хронического облучения.

В экспериментальной части статьи изучено воздействия «oral formula» (из около 30-ти антиоксидантов) и единичного парентерального агента (липосомы с плазмидой, содержащей ген MnSOD) в условиях радиационного стресса. Обнаружено, что указанные агенты снижают неблагоприятные последствия облучения. Кроме того, антитела, полученные после иммунизации животных нетоксическими дозами так называемых «радиотоксинов» (соединений, выделенных из облученных животных), также снижали последствия радиационного воздействия.

Применения антиоксидантной «формулы» на людях (пилотах) уменьшало выраженность окислительного стресса, тестируемого по ряду биохимических маркеров.

Авторы делают заключение, что их предварительные пилотные исследования могут быть важным на современном этапе не только в условиях космических полетов, но и для защиты от профессионального облучения в ядерной энергетике и при угрозе ядерного терроризма.

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Introduction

Space radiation is one of the primary environmental hazards associated with space flight. The three major sources of radiation in space are the trapped belt radiation, the galactic cosmic rays (GCR) and the solar particle events (SPE) (fig. 1). Trapped belts of energetic particles, found in the Earth's magnetic field, consist predominantly of protons and electrons. GCRs consist of protons, gamma rays and high energy, heavy (HZE) particles that originate outside the solar system. Solar flares – coronal mass ejections (CME) – are produced by solar magnetic storms that can last for hours or days. A solar particle event, which sometimes accompanies CMEs, may be the most potent space radiation hazard [1–3].

Thus, astronauts/cosmonauts on exploration class missions to the Moon, Mars, or near Earth asteroids will face acute and chronic risks of radiation from trapped particles, solar particle events and galactic cosmic rays. Ionizing radiation is recognized as a significant environmental hazard of space travel, posing a significant health risk to human crews [4–6]. Crew members are subjected to greater amounts of natural radiation in space than they receive on Earth, exposing them to immediate and long-term risks.

Space radiation can differ from gamma rays and x-rays alone, in terms of energy absorption and ionization patterns. Although a significant amount of data on biological effects of gamma rays and neutrons have been obtained from atomic bomb survivors and nuclear reactor accidents, there is very little human radioepidemiology data on bioeffects of high-energy charged particle radiation. A few studies with animals and cultured mammalian cells show that energetic protons and heavy ions can effectively induce oncogenic cell transformation *in vitro* and tumors *in vivo*. Yet, the basic mechanisms of radiation carcinogenesis remain to be clarified. Even less known are the effects of charged particles on normal tissues. Limited experimental data indicate that heavy ions

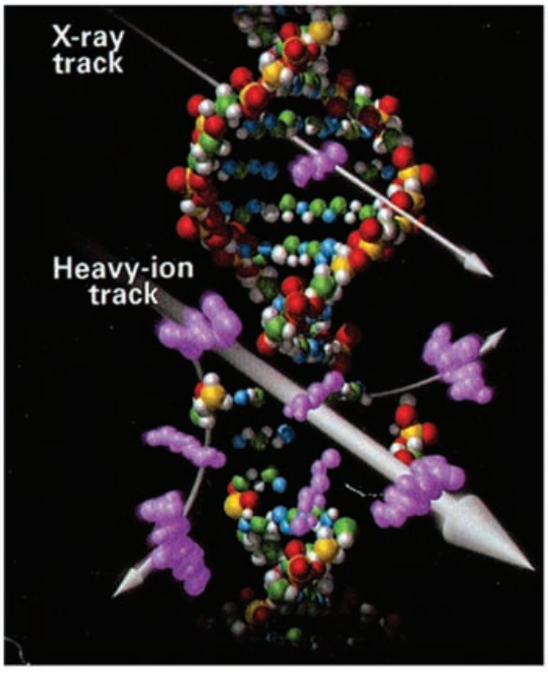

Fig. 2. Comparing DNA injury tracks between photon and relativistic heavy ion

can be more effective than gamma rays in damaging normal tissues (fig. 2). One goal of space radiation biology is the development of methods for assessment and prevention of potential radiation-induced cellular damage that could lead to cancers or other disorders during and after long-term space flights.

Considerable effort has been devoted to elucidate the biological consequences of ionizing radiation. A major mechanism of effect is the ionizing damage directly inflicted on the cells' DNA by radiation [7, 8]. Unrepaired DNA damage is known to lead to genetic mutations, apoptosis, cellular senescence, carcinogenesis, and death [9–13]. Radiation causes injury at the cellular level when ionizing particles collide directly with cellular molecules or oxidize water in a cell to form free radicals that break up

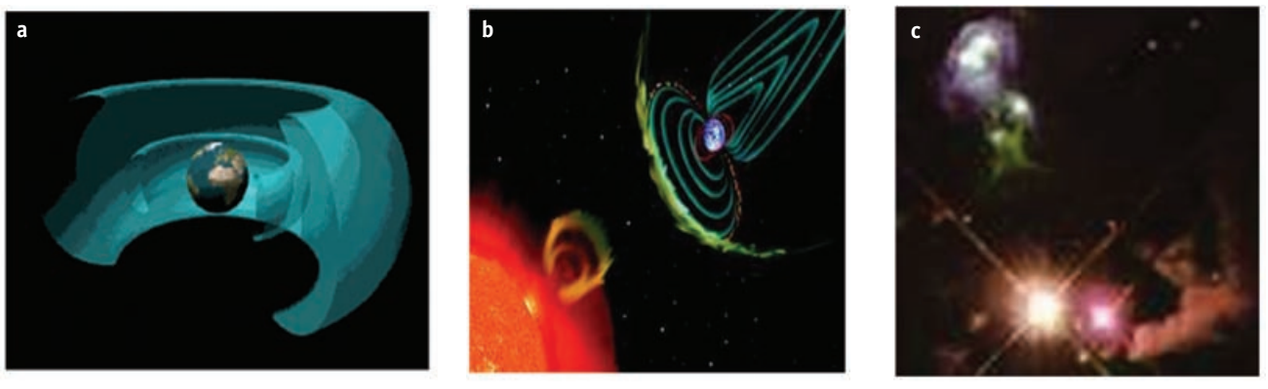

Fig. 1. Sources of space radiation: a) trapped particles in the Van Allen belts, b) Solar Particle Event from Coronal Mass Ejection distorting the Earth's geomagnetosphere, c) Supernova explosion producing GCR

or change molecular bonds. Cells are killed or altered as a result of the molecular changes. The products of acute inflammation: ROS, pro-inflammatory cytokines, adhesion molecules, prostaglandins, and compliment proteins all contribute to the progression of radiation injury leading to the symptoms of ARS. Damage to DNA molecules has particular clinical significance since alterations to the genetic blueprints of affected cells are passed onto progeny cells. While single-strand breaks in a DNA molecule can generally be repaired correctly given the double-strand redundancy in DNA structure, double-strand breaks can result in altered genes and genomic instability when full repair is unsuccessful, leading to permanent mutations in gene expression and regulation.

The ionizing effects of radiation also generate oxidative reactions that cause physical changes in proteins, lipids, and carbohydrates, impairing their structure and/or function [14, 15]. Similarly, the hydrolysis of water molecules introduces a secondary source of oxidative stress in the form of free radicals that also induce the biochemical alteration, degradation, or cross-linking of cellular macromolecules [16, 17]. Physical and functional damage to the plasma membranes and mitochondria has been reported in irradiated cells [19–21]. Known mechanisms of action of reactive oxygen species and products of lipid peroxidation including cross-linking, covalent binding to proteins, and to DNA, contribute to the toxicity of irradiation to mammalian organisms. Specific Radiation Determinant (SRD) biomolecules have newly observed toxic properties resulting from degradation, cross-linking and modification of lipids, proteins and carbohydrates [113]. The degradative enzymes, e.g. phospholipases, are activated with the oxidative stress induced by radiation. An isoenzyme of Phospholipase A2 is responsible for releasing arachidonic acid from membrane phospholipids. Phospholipase A2 exists in almost all tissues, is also found in snake venoms, and stimulates degradation of membrane phospholipids resulting in tissue necrosis. Oxidation of carbohydrates has important influence on the function of membrane glycolipids and glycoproteins in cell-cell recognition (antigen recognition) and proteins and lipids that protect the cell from the action of lipases and proteases. The radiation-induced expression of inflammatory cytokines suggests that inflammatory responses may contribute to cell death and acute radiation sickness toxicity [22]. However, the acute toxicity that is associated with ARS is not always attributed to these biological mechanisms. High dose or prolonged radiation exposure is known to increase the occurrence of cancer, cardiovascular disease, and cataracts [5, 23–25]. In addition to these long-term, degenerative consequences, acute, high dose rate exposures of radiation will induce acute radiation sickness (ARS) and death via well-defined pathologies [16]. However, the underlying cellular and molecular mechanisms that drive acute radiation-induced toxicity are not fully elucidated.

ARS Consequences [26]: An acute dose of 20 Gy or more (6–40 Gy) is considered fatal in all individuals [27], whereas 5 to 50 % lethality is expected with exposure to 2 to 3.5 Gy [28]. The prognosis of patients exposed to sub-lethal doses of radiation depends not only on the dose and dose rate received, but also the medical care available. Survival despite maximal medical care is considered unlikely for exposures greater than 10 Gy [27]. With intensive medical care – including antibiotics, blood products, and reverse isolation to prevent secondary infection – the LD50/60, which is the dose of ionizing radiation that will result in the deaths of 50 percent of the exposed population within 60 days, is 4.5 Gy, but falls to 3.4 Gy if only basic first aid is available [29].

Chronic effects of radiation exposure include progressive likelihood of neoplasia, fibrosis, and neural damage. The increased incidence of cancer is thought to be due to genomic instability, aberrant cell cycle regulation and signaling, and other mechanisms that are not completely understood [30, 31]. Skin cancer, breast cancer, leukemia, osteogenic sarcoma, thyroid and lung cancers can all develop as sequelae of radiation exposure. In addition, impaired healing and scar tissue formation may lead to cataracts; fibrosis of various organs including the heart, lungs, and kidneys; and inflammation of the gastrointestinal tract. Demyelination of the brain and spinal cord can lead to neurologic dysfunction [32]. In general, pathology depends on a number of factors such as radiation dose, dose rate, dose quality, duration of exposure, and size of the irradiated field (i.e., whole body irradiation versus focused irradiation) [2].

Radiation exposure has also been demonstrated to have adverse cognitive effects in laboratory animals. Shukitt-Hale et al. [33] found that rats irradiated with highly charged, high energy HZE particles found in galactic cosmic radiation made more errors in a maze task and exhibited cognitive decrements similar to aging. HZE particles also disrupted the dopaminergic system and adversely affected spatial learning, memory, and motor function even at relatively low doses of 0.1 Gy [2]. In humans, data are limited but prediction models suggest that exposures during large solar particle events (SPEs) are likely to impair performance. During the peak of a SPE as large as the August 1972 SPE, one of the largest on record, Hu et al. estimated that typical tasks would take 1.28 times as long as normal for completion [34].

With the above radiobiology as a background, the expectation, for crewed expeditions to the Moon and Mars, is that the radiation environment will have both general characteristics and specific distinctions that need to be taken into account when developing a system of procedures to ensure crew radiation safety. The Moon's non-existent atmosphere and the rarefied atmosphere of Mars, in combination with the absence of a magnetosphere on these planets, create an elevated radiation hazard compared with

flights in near-Earth orbits [35]. Protection from radiation during crewed expeditions to the Moon and Mars will require the development of a radiation defense system. This system should include continuous radiation monitoring on the flight trajectory and during a stay on the lunar and Martian surfaces, with the ability to predict radiation events. It should provide means and methods of reducing radiation exposure, such as a radiation shelter, emergency pharmaceutical agents, and postflight medical procedures to arrest the radiation effect [35].

Table 1 summarizes the medical manifestations expected from radiation exposure. Table 2 provides the classification of radiation injuries, while Table 3 provides the basis for classification.

There are specific operational methods that can be applied to protect astronauts and cosmonauts at the time of sporadic solar particle events (SPE) that may last from several hours to several days [26, 35]. They include intelligent planning of EVAs and moon rover excursions and the provision of a radiation shelter of adequate size. If an SPE occurs during an EVA, the time needed to reach the shelter will be a critical factor. Therefore, the timely warning of danger becomes important in defining the radius and duration of a lunar or Mars surface traverse [26, 35].

Sources of oxidative stress. Space flight inevitably increases astronauts' likelihood of cellular oxidative damage since the space environment is replete with numerous sources of oxidative stress. Some of these include high linear energy transfer radiation exposure, hyperoxic (100 % oxygen) conditions during EVA and ascent/decent, exercise, and acute gravitational stress of reentry, all of which have been associated with initiating reactive oxygen species (ROS) and oxidative damage in both human and animal studies [36–39]. Oxidative damage produces downstream effects in multiple tissue types, and since the sources are so widespread, research on oxidative damage and protection overlaps several governmental agencies and scientific groups, as can be seen in fig. 3. Countermeasures for space radiation protection could also be developed for nuclear power plant worker as well as civil populations near such sites and in cases of radio-terrorism.

Tables 1

Expected Acute General Manifestations Based on Exposure Dose (ED, in cSv* or rem of exposure) in 10, 50 and 90 % of the Population Exposed to the Dose Listed in Less than 24 Hours [16]

	Symptoms and Signs					
	Anorexia	Nausea	Vomiting	Diarrhea	Erythema	Desquamation
ED_{10}	40	50	60	90	400	1400
ED_{50}	100	170	215	240	575	2000
ED_{90}	240	320	380	390	750	2600

* cSv = centi Sievert, equivalent to 1/100 of a Sievert or 1 rem

Table 2

Classification of Radiation Injury (Based on cSv or rem of Exposure) [16]

Classification of Radiation Injury (based on cSv or rem of exposure)	
Mild (Survival Probable)	< 200 cSv
Moderate (Survival Possible)	200 cSv to 500–700 cSv
Severe (Survival Improbable)	> 700 cSv

Table 3

Basis for Classification: (LD_{50}–Lethal Dose for 50 % of the Population Exposed) [16]

Basis for Classification: (LD_{50}–Lethal Dose for 50 % of the Population Exposed)	
Blood count changes	50
Effective threshold for vomiting	100
Effective threshold for mortality	200 cSv (ED_{10}–200; ED_{50}–285, ED_{90}–350)
LD_{50} with minimal medical treatment	350 cSv
LD_{50} with supportive medical treatment	500 (480–540) cSv
LD_{50} with advanced medical treatment	1000 cSv

Evidence for oxidative stress during spaceflight.

1. Generalized markers of oxidative damage during space flight.

A number of studies show elevated levels of markers of oxidative damage among astronauts after space flight. Plasma MDA, 8-iso-prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), and

Table 4

Changes in oxidative stress biomarkers during an example ISS mission

Compound analyzed	Example Pre-flight value	Example Post-flight value	Normal ranges observed in-flight	Maximal changes observed post-flight (percentage change from pre-flight)
Total Antioxidant Capacity	1.54	1.47	1.29–1.83	Decreased up to 30%
SOD	1,318	1,172	1,092–1,817	Decreased 10–30%
Glutathione Peroxidase	51.5	50.8	27.5–73.6	Decreased 5–15%
Malondialdehyde	0.8	0.6	0–2.00	Increased 100–200%
4-OH-alkenal	0.45	0.45	0–2.00	Increased 50–150%
Urinary 8OHDG	3.2	3.7	0.49–7.29	Increased 40–200%

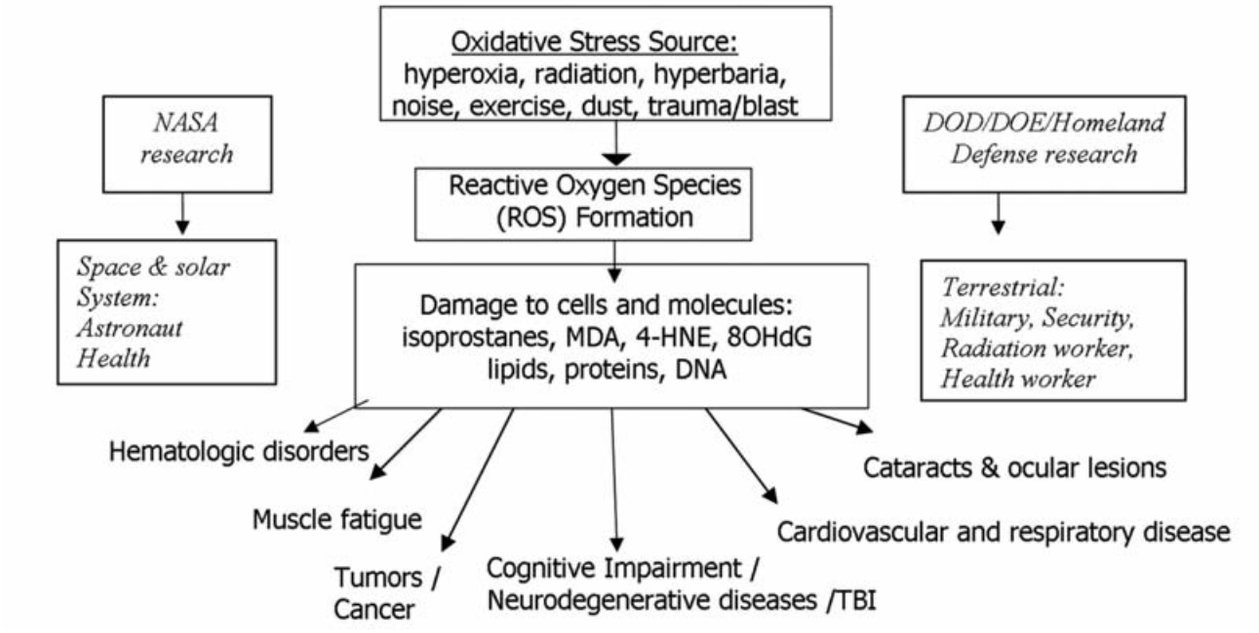

Fig. 3. Sources of oxidative stress and physiological consequences of the exposure; synergistic needs and pathways for research amongst governmental agencies and scientific communities

urinary 8-hydroxy-2'deoxyguanosine (8OHdG) have been measured during and after flight as indicators of lipid peroxidation (MDA and PGF_2) and DNA damage (8OHdG) [40, 41]. Several investigations show a significant elevation of urine 8OHdG after long-duration missions (fig. 4 a, b) but not after short-duration missions of 17 d [40, 41]. These data are supported by NEEMO data where crewmembers underwent a 14-d saturation dive where there is an increased partial pressure of oxygen [42].

Urine $PGF_{2\alpha,}$ a marker of lipid peroxidation, is decreased during flight but elevated after flight [41]. Plasma MDA is increased both during and after flight [41]. Along with increased markers of oxidative damage and decreased antioxidant defense systems, there is also a decrease in total antioxidant capacity (see fig. 5a).

Apparent increases in oxidative damage observed during and after flight could be caused by a number of factors, including altered repair mechanisms, decreased antioxidant defense systems, and increased oxidative stress. While there is not a consistently observed effect of microgravity on the repair of double-strand breaks [43, 44], there is evidence that down regulation of antioxidant defense systems occurs during space flight [45].

Another means of assessment of oxidative stress is to look at the quantity of constituent oxidative stress protection molecules pre- and post- flight. Examples include: superoxide dismutase, glutathione reductase selenoprotein family (P, W, V, S), glutathione peroxidase and thioredoxin reductase; the latter three of which require nominal selenium levels for complete enzymatic function

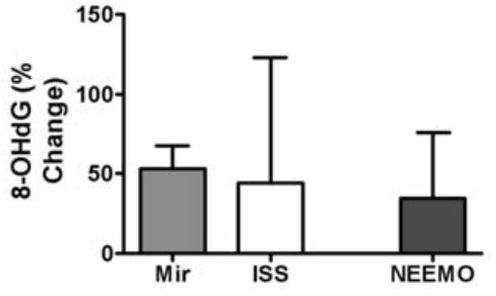

Fig. 4. The percent change of 8-hydroxy 2'deoxyguanosine (8-OHdG) from pre flight values for Mir ($n = 2$), ISS ($n = 11$) (Smith et al. 2005), and the ground based analog NEEMO ($n = 6$) [40]

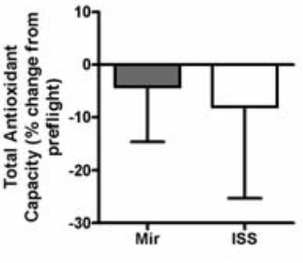

Fig. 5a. Total antioxidant capacity after space flight for Mir ($n = 2$) and ISS ($n = 11$) [42]

Fig. 5b. Urine selenium values pre- and post-flight in ISS crewmembers. (Smith S., personal communication)

(see table 4). Selenium seems to one of several micronutrients which may be depleted during long duration spaceflight, and could affect oxidative damage defense (see fig. 5b). Selenium deficiency has been associated with impaired function of the immune system [46]. Moreover, selenium supplementation in individuals who are not overtly selenium deficient appears to stimulate the immune response. In two small studies, healthy [47, 48] and immunosuppressed individuals [49] supplemented with 200 μg/day of selenium as sodium selenite for eight weeks showed an enhanced immune cell response to foreign antigens compared with those taking a placebo. A considerable amount of basic research also indicates that selenium plays a role in regulating the expression of cell-signaling molecules – cytokines, which orchestrate the immune response [50].

2. Sources of oxidative stress during space flight and/or planetary exploration.

a. Hyperoxia. Currently, astronauts are exposed to hyperoxic conditions for brief periods during launch (10–15 min), entry (30–45 min), and when they perform EVA (6–8 h). The pre-breathe protocol for U.S. astronauts typically includes 2.5-h pre-breathe of >95–100 % oxygen [26] to reduce risk for decompression sickness. After the 2.5-h pre-breathe, astronauts are typically exposed to hypobaric 100 % oxygen for 6 to 8 hrs during EVA. The literature is replete with studies showing injury to virtually all organ systems following exposure to hyperoxia or radiation [16, 51]. A hyperoxic environment can induce oxidative damage and impair antioxidant capacity, as demonstrated in numerous ground-based experiments using both normobaric and hypobaric conditions. Under physiological conditions (i.e., 21 % O_2), approximately 2–3 % of the oxygen consumed by the body is converted into oxygen-derived reactive oxygen species [52].

Human antioxidant defenses are designed to protect the body in 21 % oxygen environments, but these defenses are easily overwhelmed under hyperoxic environments. It was first suggested in the 1950s that a hyperoxic environment may be toxic based on eye damage among premature infants in incubators with high oxygen concentration [37–39]. Evidence exists for increased lipid peroxidation after acute (2 h) >95 % oxygen exposure. Increased lipid peroxidation (measured by urinary n-pentane), occurs in humans within 30 min of breathing 100 % O_2 [53]. In another study, elevated plasma malondialdehyde (MDA, another index of lipid peroxidation) was reported in healthy humans after 125 min of normobaric100 % oxygen exposure [54]. Animal studies support the human data [55, 56]. While the accuracy of n-pentane as a marker of lipid peroxidation is debated [57, 58], but increased n-pentane and MDA provide clear evidence that lipid peroxidation increases during hyperoxia. Furthermore, hyperoxic conditions are

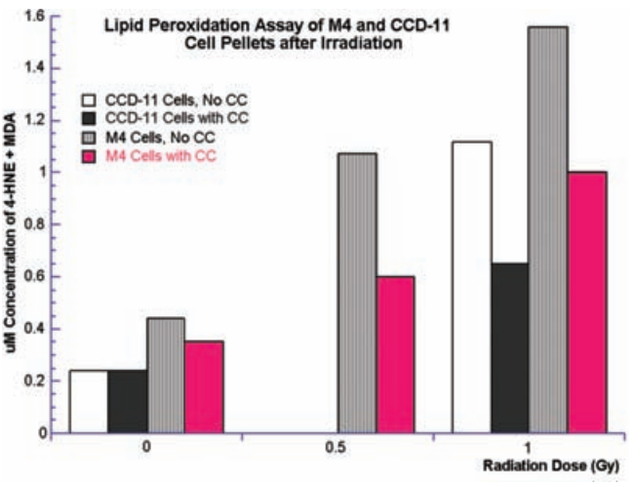

Fig. 6. Measures of *in vitro* lipid peroxidation in lung (CCD-11) and breast (M4) cells exposed to HZE ionizing radiation, a component of galactic cosmic rays

also found to induce vasoconstriction in humans [59], deplete pulmonary extracellular superoxide dismutase (SOD) in mice [60], and increase apoptosis in PC12 cells [61], all of which indicate that hyperoxia can induce cellular oxidative damage.

Antioxidant status also decreases during hyperoxic conditions. For example, during acute (3 h) normobaric hyperoxia [62] in birds, serum antioxidant (e.g., alpha- and gamma-tocopherol, carotenoids) concentrations were decreased. Hepatic vitamin E stores was also reduced in preterm guinea pigs exposed to 98 % oxygen for 48 h [63]. Unlike space flight, however, the activity of several antioxidant enzymes (e.g., SOD, glutathione peroxidase, glutathione reductase, catalase) increase in animal studies of acute hyperoxia [64, 65].

b. Radiation Exposure. Astronauts flying to high altitudes (e.g. ISS, Hubble telescope repair), and beyond the geomagnetosphere, also have the impact of higher space radiation exposure. Cytogenetic biodosimetry assesses the biological impact of radiation exposure, and it involves harvesting white blood cells and quantitating numbers of chromosomal aberrations, DNA strand breaks, and translocations. Other biodosimetry tools allow for correlation of the physical dose as measured by crew dosimeters and the quality factor of the radiation as approximated by the Tissue Equivalent Proportional Counter. Increases in chromosomal aberrations and DNA damage after space flight are well documented Damage to cellular components such as DNA is a complex process and includes both direct damage from high-energy particle impacts on the molecules themselves, as well as indirect damage from the production of ROS [16, 51].

Supporting evidence of the oxidative stress experienced by spaceflight crewmembers has been observed in numerous *in vitro* and rodent experiments which were

exposed to "space-like radiation" i.e. high energy particle HZE radiation at particle beam accelerator facilities in the US and Japan [66]. In these studies markers of oxidative stress such as MDA and 4-HNE (lipid peroxidation, fig. 6) and 8OH-DG (DNA adduct) were elevated, transmembrane potential was altered (lipid damage due to peroxidation), and chromosomes/nucleic acids were damaged as revealed by FISH and COMET assays showing breaks, mutations and ploidy disturbances [66]. Additional evidence reveals that space radiation induces oxidative damage that results in the increased incidence of cataracts observed in astronauts traveling outside the geomagnetosphere or exposed to HZE radiation with high altitude low Earth orbit missions [67].

c. Interaction with planetary regolith. Through robotic and Apollo mission analysis of regolith as well as curation-facility biochemistry of lunar derived soils, scientists and toxicologists have identified inhaled regolith as a potential hazard to astronauts performing EVA on the lunar surface. Lunar regolith is composed of approximately 40 % silica, most of which is in an amorphous state, and relatively non-reactive with human tissue. However, freshly cut or crystalline silica, especially in the < 10 micron size range, can be quite toxic because of dissemination into the lung periphery with alveolar trapping and entry into the lung interstitium. Free radicals and superoxides are generated in the lung upon interaction of macrophages and other cellular immune cells with the silica particles, damaging bystander pneumocytes. Pulmonary damage may be the direct consequence of toxic interaction between quartz particles and cell membranes, or may be due to silica-induced production of oxidant species by pulmonary phagocytes, that in turn overwhelms pulmonary antioxidant systems and causes lung injury [68–70]. Data indicate that grinding or fracturing quartz particles breaks Si-O bonds and generates •Si and Si–O• radicals on the surface of the cleavage planes. Upon contact with water, these silica-based radicals generate hydroxyl radicals (•OH) (fig. 4). These surface radicals decay as fractured silica dust ages. Freshly fractured quartz is significantly more potent than aged silica in directly causing lipid peroxidation, membrane damage, and cell death. This silica-induced activation results in the production of superoxide (O_2^-), hydrogen peroxide (H_2O_2), nitric oxide (NO•), and other oxidant species that can damage lung cells [38–40]. The ultimate consequence of silicosis is pulmonary fibrosis, which can be severely debilitating or even lethal if the exposure is severe and/or of long duration [71–78].

d. ROS Generation during Exercise. Exercise-induced fatigue and muscle atrophy are also mediated in part by ROS. Electron spin resonance spectroscopy technology confirmed earlier findings from the 1950s suggesting that short-lived reactive intermediate molecules like ROS are present in skeletal muscle after exercise. Since then, numerous studies support a role of ROS in skeletal muscle fatigue. ROS denature proteins directly associated with the sarcoplasmic reticulum Ca^{2+} release mechanism, thus compromising tension development. Furthermore, decreased antioxidant status lowers exercise capacity and increases onset of fatigue in human and animal studies [79]. Astronauts perform extensive upper body exercise during EVA activity, and one of the limiting factors in completing EVA tasks is forearm and hand muscle fatigue due to extensive tool operation. The fatigue often requires crewmembers to stop and rest, thereby prolonging the duration of EVA and limits the number of tasks performed during each EVA [80].

e. Monitoring radiation injury and oxidative stress. As a step toward that goal, researchers are developing newer methods for evaluating the bioeffects of radiation injury. These methods, in addition to traditional physical "badge-type and instrument" dosimetry, may include biomarkers of exposure: e.g. transepithelial membrane resistance, measurement of membrane sheer, products of lipid peroxidation, and DNA-adduct formation. Other possible methods may evaluate markers of health risk, including e.g. assessing cellular DNA for chromosomal aberrations, breaks, and translocations, plus dicentrics, micronucleus formation, etc. via chromosomal painting, FISH, PCC, COMET, γH2AX analysis, flow cytometry and newer methods e.g. genomic or proteomic profiling. For exploratory class missions of long duration, tools for pre-flight risk assessment and in-flight monitoring of oxidative stress and cellular injury will be invaluable to along the crew to modulate their biological exposure and their employment of shielding and countermeasures.

Employing an immune enzyme assay is a very efficient tool for biological dosimetry and evaluation of the differential diagnosis of acute radiation disease**.** The immune assay targets biological markers of radiation toxicity – high molecular weight glycoproteins with specific antigenic properties, found specifically in association with the hematopoietic, cerebrovascular, cardiovascular and gastrointestinal forms of acute radiation syndromes (ARS). The important goal of an early assessment with the enzyme immune assay is the accurate description of the ARS at the initial phases. Early and precise differential diagnosis allow physicians to provide an effective medical management of ARS.

Countermeasures Development. Because acute radiation sickness occurs within a very short period of time, the opportunities to treat or mitigate the effects of high-dose irradiation are very limited. As an augmentation to treatment, a prophylactic measure could be a more effective strategy to address this acute radiation-induced phenomenon. In addition, preventing the onset of ARS may also be beneficial in minimizing the other biological consequences of ionizing radiation. For this desired effect,

the authors will describe the development of an oral formula or "cocktail", directed at reduction of oxidative stress, and enhancement of inherent cellular defense mechanisms. It is likely, if the formula was non-toxic to the crewmember, that such an approach could also be employed for reducing the damage associated with chronic low-dose radiation exposure as well. Secondly, we will describe the development of a previously untested parenterally administered agent, to augment the cell's inherent defenses against high dose oxidative injury, e.g. from a high dose rate, acute radiation exposure. The authors have previously described a novel biological mechanism of acute radiation toxicity that originates in the lymphatic system, associated with novel radiotoxins that appear in radiosensitive tissues after irradiation, called specific radiation determinants (SRD) [81]. Thus thirdly, the authors will summarize the development of an experimental anti-radiation vaccine against these SRD's which, because it is directed at a biological mechanism other than DNA damage or oxidative stress, this immunologically based form of prophylaxis may be a powerful adjunct therapy that will enhance the efficacy of existing and proposed radiation countermeasures.

This collaboration has pursued 3 areas of countermeasures development: 1) acute effects mitigation, 2) late effects reduction and 3) oxidative stress modulation / prevention. The work has progressed over a decade and began with cell culture experiments, has progressed to multiple animal studies, and has included preliminary human studies.

Oral Agents: Rationale for development of a chemopreventive / oxidative stress protective formula:

Certain antioxidants, e.g., α-tocopherol, ascorbic acid, beta-carotene, SOD, glutathione peroxidase, catalase have properties that protect cells from oxygen free-radical toxicity [82], and therefore have the potential to decrease the type of oxidative damage observed among astronauts that may be caused by hypobaric hyperoxia, and may also be able to reduce oxidative damage associated with prolonged hyperoxic environments. Vitamin C is a potent antioxidant capable of reversing endothelial dysfunction caused by increased oxidant stress [83]. Though it seems likely that vitamin C supplementation would mitigate hyperoxia-induced oxidative damage among EVA, it is debated whether vitamin C could act as a pro-oxidant when iron stores are elevated [84, 85]. In one study, treatments with vitamin A, C, or E protected rats exposed to acute hyperoxia (80 % oxygen) against oxygen toxicity by elevating glutathione concentration [86]. In another study, vitamin E supplementation to rabbits decreased lipid peroxidation and diminished increases in pulmonary antioxidant enzymes induced by *in vitro* 100 % oxygen exposure [65]. These increases likely contribute to symptoms of oxidative stress. In another *in vitro* study, α-tocopherol was effective in preventing hyperoxia-induced DNA fragmentation and apoptosis [61]. Flavonoids have been found to exhibit more antioxidant effects than α-tocopherol in healthy adults, but these compounds have never been tested against hypobaric hyperoxia-induced oxidative damage [87]. In addition to a plethora of other tested agents, e.g. a-lipoic acid, folic acid, co-enzyme Q10, selenium, beta carotene, glutathione, and N-acetylcysteine, there are a large number of plant extracts that have been investigated for their antioxidant properties, such as strawberry and blueberry, hawthorn, *Periplaneta americana*, and curcumin [26].

The FDA's approval of a cardiovascular health claim for nutritional products containing 25 gm of soy protein has contributed to widespread use of soy supplements. Kaplan's monkey study [88] indicates that long-term consumption of soy protein containing a modest amount of isoflavones inhibits the early progression of coronary artery atherosclerosis. In addition to reducing the risk of heart disease, isoflavones are being studied in relation to the relief of certain menopausal symptoms, cancer prevention, and slowing or reversing osteoporosis. Biochemical studies conducted in mice at Johns Hopkins (Nathan Congdon, ARVO 2003) indicate isoflavones (Genestein) potential in the prevention of oxidative damage leading to cataract. No. such data is available in human or non-human primates. The anti cataract drug OT 551, (Tempol H) a powerful antioxidant, to prevent lens protein aggregation, and thus can serve as a countermeasure for lens protein damage leading to cataract, on astronauts exposed to cosmic radiation [89]. Other oral antioxidant formulas are now standard of care in prevention of macular degeneration [6]. Quercetin, a plant bioflavanoid, has shown itself to be a powerful antioxidant and free radical scavenger while also demonstrating anti-carcinogenic, neuroprotective, anti-viral, and cardio/vascular protective properties. It has also been shown to help prevent cataract formation and exhibit positive effects on cognitive performance and immune response [90, 91, 93]. *In vitro* experiments suggest it may also be beneficial in protecting against bone loss. Furthermore, recent studies funded by DARPA (Defense Advanced Research Projects Agency) have suggested a protective mechanism against viral illness after exertional stress in athletes and synergistic properties with other micronutrients such as Vitamin C, B3, and omega-3 fatty acids [91–95].

Studies performed by Lupton, Turner and colleagues with the NSBRI show potential reduction in cancer risk in animals exposed to carcinogens and ionizing radiation when supplemented with omega-3 fatty acids and fiber [96]. Omega-3 fatty acids have also shown benefit in improving cholesterol and lipid parameters in those with unfavorable total cholesterol to high density lipoprotein ratios [97, 98]. The safety and efficacy of using algal source

omega-3 supplementation, compared with other sources, such as fish has been shown in multiple studies [99, 100]. Combinations of DHA and EPA, and other fatty acids are beginning to also show efficacy in improving cognitive performance and mood, in test subjects with affective disorders, traumatic brain injury or exposed to environmental stress [102–110].

Thus it was postulated, that a formula mixing low levels of each of the most effective protection molecules, allows delivery to the human without the toxicity associated with high-dose, single agents, and with conceivably better efficacy [101].

Parenteral Treatment of radiation toxicity: In addition to the potential employment of parenteral agents such as Amifostine (WR-1065) in reducing nephrotoxicity due to acute radiation exposure, current medical management is based on cellular component replacement and supportive therapy. Hematopoietic cell transplantation has been recommended as an important method of treatment of the hematopoietic form of ARS. However in several different hospitals and institutions, 31 patients with the hematopoietic form of ARS received stem cell transplantation and, in all cases (100 %), the transplants were rejected, and the lethality rate was 87 %. Thus new and innovative approaches are necessary to improve the outcomes in high dose acute radiation exposures.

Material and Methods

In vitro studies. The formula was initially tested in cell culture with gamma and HZE particulate radiation from accelerators in NYC, NY and Chiba, Japan and found to show promise in reducing lipid oxidative damage and DNA lesions [66].

In vivo, Animal studies. (Oral + parenteral countermeasures) C57BL/6NHsd mice receiving intravenous MnSOD-PL prior to 9.5 Gy total body irradiation showed increased survival from the acute hematopoietic syndrome and males demonstrate improved long term survival [111]. So then, based on pilot data, suggesting possible synergy with parenterally administered superoxide dismutase-containing liposomes, the formula was tested in rodents at the University of Pittsburg.

Study 1 M&M: Evaluation of whether an antioxidant-chemopreventive diet compared to a regular diet improved long-term survival in female mice. C57BL/6HNsd female mice (18 to 20 gm) were housed 5 per cage and maintained according to IACUC protocols. 160 female C57BL/6NHsd female mice (8 weeks of age) which were divided into 4 groups of 40 mice. Twenty-four hours before the LD 50/30 dose of 9.5 Gy TBI subgroups of mice were injected intravenously with MnSOD-PL (100 μg plasmid DNA in 100 μl). Two of the groups were placed on the antioxidant-chemopreventive diet (table 1) 7 days before irradiation and maintained on the diet until conclusion of the experiment. The other two groups were maintained on the regular or "house" diet (LabDiet rMH 3000 (5P00) with 0.12 % hydrogen silicon dioxide from TestDiet, catalog #1812877). The silicon dioxide is added as an inert compound to compensate for weight changes due to addition of antioxidant ingredients. The antioxidant diet consisted of a micronutrient multivitamin and trace mineral formula ("AmeriSciences®/NASA Premium Multivitamin Premix", AmeriSciences LP, Houston, TX) and a non-essential antioxidant and chemoprevention mixture derived primarily from natural foods ("AmeriSciences®/NASA Fruit and Veggie Antioxidant Formula Premix", AmeriSciences LP, Houston TX). Of this chow serving size, 99.95 % was chow mix, 0.024 % was the AS/NASA Premium Multivitamin Formula (table 1), and 0.023 % was the AS/NASA Fruit/Veggie Antioxidant Formula. The constituents of the antioxidant and chemoprevention diet supplements are shown in table 5 [112].

In vivo, Animal studies. Several studies were conducted at the Moscow Veterinary Academy and other locations, evaluating the efficacy of both hyperimmune serum and a parenteral vaccine on survival of animals receiving an ^{9d}LD100 dose of total body irradiation.

Study 2 M&M: The following experimental animal species were studied: mice, rat, rabbit, sheep, pigs, dogs and cattle. All animals possessed normal blood profiles, weight and size for age, and body temperatures. The animals were exposed to gamma rays, based on body weight of the animals in doses up to 10 Gy, and were irradiated in RUM-17, Puma, and Panorama devices. The exposure dose rate ranged from 3–29 A/kg. On the day preceding radiation exposure, and also 15, 30, and 45 days post-exposure, a lympho-venous anastomosis was created surgically. Mild, moderate, severe, and extremely severe acute radiation sickness of the hematological form, as well as the gastrointestinal, toxic and cerebral acute radiation syndromes, were induced in the experimental groups of animals. Gel filtration and high-performance liquid chromatography were used to extract the immunochemical glycoprotein specific radiation determinants (SRD) from the central lymph of animals. SRD (Specific Radiation Determinant) radiation toxins have been analyzed and found to be glycoproteins with the molecular weight ranging from 200–250 kDa and with high enzymatic activity.

The vaccine was produced from lyophilized SRD (isolated from the lymph of animals irradiated at doses inducing cerebral and extremely severe ARS), which were dissolved in an isotonic solution of NaCl. The dose of administered was based on computation of the amount of SRD per unit volume of central lymph and absorbed dose of radiation. Animals were randomly assigned to receive placebo, vaccine or hyperimmune serum before exposure to radiation. The animals received subcutaneous injections

Table 5

Constituents of the rodent chemoprevention test formula, used with and without the Mn-SOD plasmid liposomes

MICRONUTRIENT COMPONENTS:	Daily dose per mouse†	Equivalent Human Daily Dose	Human UL* (19-70 yo group)	Human NOAEL**
Vitamin A (30% as vitamin A palmitate and 70% as beta-carotene)	0.2451 IU	750 IU	10,000 IU	10,000 IU
Beta-carotene (part of Vitamin A total)	0.3431 mcg	1.05 mg	NE‡	25 mg
Vitamin C (as ascorbic acid)	0.0817 mg	250 mg	2000 mg	>1000 mg
Vitamin D (as cholecalciferol)	0.3921 IU	1200 IU	4000 IU	800 IU
Vitamin E (as d-alpha tocopheryl succinate and mixed tocopherols)	0.0653 IU	200 IU	1490 IU	1200 IU
Vitamin K (as phytonadione)	0.0261 mcg	80 mcg	NE	30 mcg
Thiamine (vitamin B1) (as thiamine mononitrate)	0.7352 mcg	2.25 mg	NE	50 mg
Riboflavin (vitamin B2)	0.8332 mcg	2.55 mg	NE	200 mg
Niacin (as inositol hexanicotinate)	9.802 mcg	30 mg	35 mg	500 mg
Vitamin B6 (as pyridoxine hydrochloride)	0.9802 mcg	3 mg	100 mg	200 mg
Folate (as folic acid)	0.1960 mcg	600 mcg	1000 mcg	1000 mcg
Vitamin B12 (as cyanocobalamin)	0.0029 mcg	9 mcg	NE	3000 mcg
Biotin	0.1470 mcg	450 mcg	NE	2500 mcg
Pantothenic acid (as d-calcium pantothenate)	4.901 mcg	15 mg	NE	1000 mg
Calcium (as calcium carbonate, dicalcium phosphate)	0.1634 mg	500 mg	2500 mg	1500 mg
Iodine (from kelp)	0.0098 mcg	30 mcg	1100 mcg	1000 mcg
Magnesium (as magnesium oxide and chelate)	65.35 mcg	200 mg	350 mg	700 mg
Zinc (as zinc chelate [monomethionine])	4.901 mcg	15 mg	40 mg	30 mg
Selenium (as L-selenomethionine)	0.0327 mcg	100 mcg	400 mcg	200 mcg
Copper (as copper amino acid chelate)	0.0588 mcg	0.18 mg	10 mg	9 mg
Manganese (as manganese amino acid chelate)	0.6535 mcg	2 mg	11 mg	10 mg
Chromium (as chromium polynicotinate)	0.0653 mcg	200 mcg	NE	1000 mcg
Molybdenum (as molybdenum amino acid chelate)	0.0183 mcg	56 mcg	2000 mcg	350 mcg
Potassium (as potassium citrate)	94.75 mcg	290 mg	NE	NE
Choline (as choline bitartrate)	16.34 mcg	50 mg	3500 mg	NE
Inositol (as inositol and inositol hexanicotinate)	16.34 mcg	50 mg	NE	NE
Boron (as boron chelate)	0.3267 mcg	1 mg	20 mg	NE
Vanadium (as vanadyl sulfate)	0.0163 mcg	50 mcg	1800 mcg	NE
NON-ESSENTIAL NATURAL ANTIOXIDANT & CHEMOPREVENTION AGENTS:				
Rutin	8.036 mcg	25 mg		
Quercetin	257.1 mcg	800 mg		
Hesperidin	1.607 mcg	5 mg		
Alpha Lipoic Acid	128.6 mcg	400 mg		
N-Acetyl-L-Cysteine (NAC)	192.9 mcg	600 mg		
Lutein	3.214 mcg	10 mg		
Lycopene	1.607 mcg	5 mg		
Astaxanthin	0.3214 mcg	1 mg		
Plant Sterols	80.36 mcg	250 mg		
Isoflavones (from soy extract)	8.036 mcg	25 mg		
Garlic Extract (bulb)	88.39 mcg	275 mg		
Green Tea Extract (leaf)	80.36 mcg	250 mg		
[standardized to 95% polyphenols and 50% epigallocatechin gallate (EGCG)]				
Cruciferous Vegetable Extract (Brassica spp.) (plant)	32.14 mcg	100 mg		
Fruit Blend	32.14 mcg	100 mg		
(strawberry, escobillo, blueberry, blackberry, cranberry, grape, pomegranate)				
Ginkgo Biloba Extract (leaf)	19.29 mcg	60 mg		
Coenzyme Q-10	32.14 mcg	100 mg		
Resveratrol	1.607 mcg	5 mg		

of an anti-radiation vaccine in doses of 5, 10, 15, or 20 μg/kg lean mass and the control animals were injected with 1.5 ml of normal saline solution; 10, 15, 30, 60 and 90 days before irradiation. The animals received lethal doses of radiation 15, 30, or 60 days after vaccination. The criterion for vaccine efficacy was survival of the animals 30 days after irradiation: for rats at a dose of 10.0 Gy, rabbits at a dose of 9.5 Gy, and dogs at dose of 6.5 Gy [81].

In vivo, Human studies. Several small pilot studies have been conducted to test the tolerance and efficacy of a formula countermeasure. The studies were based on effects observed during NEEMO V, XII and XIII missions used to determine whether there was indeed evidence of oxidative stress during the mission, a 2 week NEEMO saturation dive [42]. Along with the increased 8(OH) dG excretion during the dive, decreased activities of GPX and SOD during (SOD) and after (GPX and SOD) the dive, imply that oxidative stress and inflammation increased [42].

Study 3 M&M: A follow-up pilot study was performed to assess muscular fatigue reduction with a NAC-based countermeasure formula. The study was conducted on crewmembers training in the NBL during 6–8 hour hyperoxic environmental exposures, and examined their ability to perform tasks observed to induce forearm fatigue during EVA training activities.

During Neutral Buoyancy Laboratory (NBL) spacewalk training dives, employing Nitrox (approx. 40 % oxygen-enriched dual (O2/N2) gas mixture– exposing crewmembers to UPTD of approx. 1300; lipid peroxidation markers were measured pre- and post- dive. On one dive the crewmember received no countermeasure formula, on the other dive the crewmembers received the countermeasure beginning 1 week prior to the dive.

Results

Study 1 R: MnSOD-PL administration improves survival from LD 50/30 total body irradiation:

Mice that received intravenous administration of 100 μg of plasmid DNA in 100 μl of liposomes showed improved survival as compared to mice in the control group after 9.5 Gy TBI. MnSOD-PL showed increased survival from the acute effects of 9.5 Gy TBI ($p = 0.031$) [112]. Mice receiving the antioxidant diet alone did not show an improvement in survival at 30 days with a percent mortality of 50 % compared to 45 % for the control diet ($p = 0.82$). The data confirm the previous publication [111] and demonstrated decreased 30-day mortality in the MnSOD-PL group as compared to the control: 20 % mortality in the MnSOD-PL group vs. 45 % in the control ($p = 0.031$). Thirty-day mortality was significantly lower in the antioxidant diet + MnSOD-PL group compared to the control house diet or antioxidant diet only: 17.5 % for the antioxidant diet + MnSOD-PL group vs. 45 % mortality in irradiated house diet controls and 50 % in the

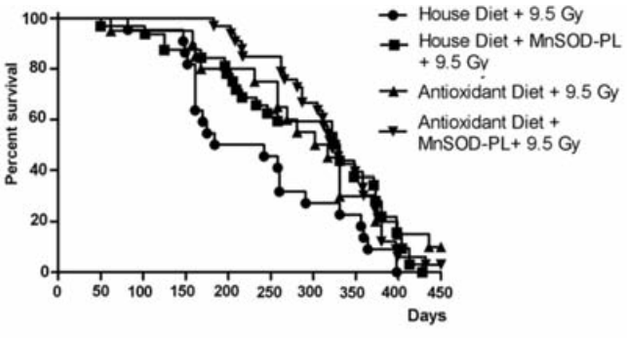

Fig. 7. Conditional survival curves (animals surviving at least 30 days) for each of four test groups receiving 9.5 Gy total body irradiation [112]

antioxidant diet ($p = 0.015$ and 0.0041 respectively) [112].

Antioxidant diet improves conditional survival and ameliorates radiation-induced life shortening:

Mice surviving the 9.5 Gy total body irradiation dose at 30 days were followed for evaluation of the late effects of total body irradiation (conditional survival). As shown in fig. 7, the conditional survival of mice on the antioxidant diet was significantly improved over the 450 days of observation compared to those on the house diet ($p = 0.040$). Mice on the antioxidant diet that received MnSOD-PL in addition, also showed an improvement in conditional survival compared to those on the house diet alone ($p = 0.010$, fig. 7) [112]. These results establish that antioxidant diet supplements ameliorate radiation-induced life shortening and provide support for the concept of continuing oxidative stress in the post-irradiation cellular microenvironment of tissues, organs and organ systems [112].

Study 2 R: Administration (I/V or IM) of SRD RT to healthy, radiation naïve animals induced the development of clinical symptoms of ARS. Administration of the RT SRD-1 (doses ranging: 0.1, 0.5, 1, 10, 30, 50, 70 and 100 mg/kg) to non-radiated animals induced acute toxicity which compares to the deleterious effects generated by high doses irradiation [113]. The highest doses of RT produced death of radiation-naive animals within hours to days after administration of toxins. For these animals injected with SRD-1 toxins, a short period of extreme agitation was followed by deep coma, and subsequent circulatory and respiratory depression. The results of postmortem histology showed characteristics of intra-cortical hemorrhage and other effects similar to high dose acute cerebral irradiation [113, 114].

Antibodies raised against the SRD antigens can reduce or neutralize the toxic properties of RT-SRD administration, as well as reduce the toxicity associated with acute high dose irradiation (see table 6).

Serum containing anti-radiation antibodies cans also be an effective method for decreasing radiation toxicity and detoxification of these agents, with similar effects to the vaccine, but of limited duration utility (data not published) [114].

From these studies, it would seem that an anti-

Table 6

Summary of the effect of high dose radiation (expressed in Gy) on various animal species and the impact of a radiation vaccine countermeasure [113]

Species	Radiation (Gy)	SDR vaccine (mg/kg)	Number of animals	Survival rate (%) 30 days	60 days	180 days	360 days
Dogs	6.5	0	17	0	0	0	0
		15	93	88	79	65	65
Pigs	7.5	0	30	0	0	0	0
		15	68	65	61	54	54
Sheep	6.5	0	23	0	0	0	0
		20	112	90	84	78	78
Horses	6.5	0	5	0	0	0	0
		20	19	14	13	13	13
Cattle	9.2	0	10	0	0	0	0
		20	60	59	57	54	51
Rats	8.5	0	250	0	0	0	0
		10	3696	3326	3142	---	---
Mice	7.0	0	300	0	0	0	0
		10	2170	1628	1628	---	---

radiation vaccine and an anti-radiation immune IgG serum preparation can be effective in diminishing the development of post-radiation burns and improve clinical symptoms of combined radiation injury. Once immunized, due to immunological memory formation, the animals are able to maintain a reasonably high level of resistance to radiation for several years. Thus, anti-radiation serum and vaccine, could be considered as a part of a radioprotection strategy to assist military forces to operate in radioactive zone of military operations, and to protect civilian population in areas of nuclear plant accidents or terroristic attack with nuclear weapon use.

Study 3 R: The following were evaluated: the magnitude of oxidative stress in EVA crewmembers, as measured by markers of lipid peroxidation, DNA damage, and total oxidant capacity; the efficacy of antioxidant countermeasures in reducing simulated EVA-induced total oxidative stress; and the efficacy of an antioxidant countermeasure to reduce muscular fatigue seen during EVA-type activities during NBL training. Each graph in fig. 8A reveals the changes in either cellular protection molecule (e.g. SOD) or oxidative stress marker (e.g. MDA, 4HNE) associated with exposure to hyperbaric oxygen during an 8-hour NBL Nitrox, hyperoxic training run.

The crewmembers served as their own controls in this pilot study experimental design. In general, there was less lipid peroxidation and better hand-grip endurance when crewmembers were taking the countermeasure formula.

After completing the NBL pilot study, several astronauts, by their own request, have taken the chemoprevention formula during both short duration flight on the Space Transportation System (Shuttle) orbiter and the International Space Station (ISS). The formula was well tolerated pre- and in-flight by all four crewmembers, and there were no in-flight side effects of the formula (personal communication, data not published). The content of the human spaceflight formula is shown in table 7.

Discussion

There are many sources of oxidative stress in the lives of workers, whether they work in nuclear power facilities, on the front lines of international conflicts, or in the reaches of outer space. The exposure dose can vary substantially, but at minimum will accelerate the aging of their organ systems, and at worse could result in acute exposure syndromes that may be fatal. A common thread of the oxidative stress exposures is ROS-binding to critical cellular organelles and molecules, which can result in cellular dysfunction, mutation of nucleic acids, or even apoptotic cell death [30]. Currently there are no proven countermeasures for these exposures, aside from a clinical agent, Amifostine, (Etyhol™), which is used to reduce mucositis and other side effects from radiation therapy dose in cancer patients [26]. This manuscript describes: 1) sources of oxidative stress during spaceflight, 2) the complexity of the radiation exposure outside of the Earth's geomagnetosphere, and 3) some potentially fruitful avenues of research in developing prevention, mitigation and treatment strategies for those who are occupationally exposed to excessive sources of oxidative stress, especially acute and chronic radiation.

The cytotoxic effects of different types of radiation may be the single most important clinicopathologic process by which oxidative damage is induced from reactive oxygen species and radiation toxicity induced by radiation toxins. Radiation toxins (SRDs) with high enzymatic activity and their ability to degraded a wide variety of extracellular proteins, lipids, carbohydrates and DNA molecules, induce damage of important intracellular compartments such as mitochondria, ion channels, DNA, as well as activating degradation of peptide bonds in important polypeptides in tissues and vascular endothelium. Yet the exact mechanism by which radiation toxins stimulate development of the ARS is poorly understood. SRD radiation toxins possess both antigenic and toxic properties; yet the antigenic properties can be utilized to

Table 7

Constituents of human spaceflight chemoprevention formula, flown on Shuttle, ISS flights

	Daily Dose	Unit
A.) Multivitamins/Trace Minerals (as tablet)		
Vitamin A (as 70 % beta-carotene and 30 % vitamin A palmitate)	2500	IU
Vitamin C (as ascorbic acid)	250	mg
Vitamin D (as cholecalciferol)	1200	IU
Vitamin E (as natural d-alpha tocopherol succinate and mixed tocopherols)	200	IU
Vitamin K (as phytonadione)	80	µg
Thiamine (vitamin B1) (as thiamine mononitrate)	2.25	mg
Riboflavin (vitamin B2)	2.55	mg
Niacin (as inositol hexanicotinate)	30	mg
Vitamin B6 (as pyridoxine hydrochloride)	3	mg
Folate (as folic acid)	600	µg
Vitamin B12 (as cyanocobalamin)	9	µg
Biotin	450	µg
Pantothenic acid (as d-calcium pantothenate)	15	mg
Calcium (as calcium carbonate, dicalcium phosphate)	500	mg
Iodine (from kelp)	30	µg
Magnesium (as magnesium oxide and chelate)	200	mg
Zinc (as zinc chelate [monomethionine or glycinate])	15	mg
Selenium (as L-selenomethionine)	100	µg
Copper (as copper amino acid chelate)	0.18	mg
Manganese (as manganese amino acid chelate)	2	mg
Chromium (as chromium picolinate)	200	µg
Molybdenum (as molybdenum amino acid chelate)	56	µg
Potassium (as potassium citrate) (7.5 mEq)	290	mg
B.) Antioxidant/Chemoprevention agents (as capsule)	Daily Dose	
Quercetin [Source quercetin dihydrate and/or citrus peel)]	800	mg
Rutin/Hesperidin Source citrus peel]	25/5	mg
Green Tea Polyphenols [Source: Green Tea Extract (leaf)]	450	mg
Epigallocatechin Gallate (EGCG)	250	mg
Alpha Lipoic Acid	100	mg
N-Acetyl-L-Cysteine(NAC) synthetic	600	mg
Lycopene [Source: Source: Tomato Extract 5 %]	5	mg
Astaxanthin [Source: Haematococcus Algae Extract 2 %] Lutein Source [Source: Marygold Extract 5 %]	1 10	Mg mg
Phytosterols [Source: Soy and Avocado]	250	mg
Isoflavones [Source: Soy and/or Avocado Extracts]	350	mg
Allicin [Source: High-Potency Garlic Extract (bulb)]	7.5/275	mg
Glucosinolates [Source: Cruciferous Vegetable Extract (Brassica spp.) (plant)]	4/100	mg
High ORAC Fruit Extract [Source: strawberry, escobillo, blueberry, blackberry, cranberry, grape, pomegranate]	1000	mg
Coenzyme Q-10	100	mg
Resveratrol [Source: phytoalexin from grape juice/seed extract (incl: flavonoids, polyphenols, proanthrocyanins)]	150	mg
Lipid Supplement (from omega-3 fatty acids alpha-linolenic, as gel capsule)		
DHA (docasahexaenoic acid– from algal oil) EPA (eicosapentanoic acid– from fish oil)	1500 500	mg mg

neutralize the toxic properties, by inducing specific antibodies which limit SRD toxicity [113, 114].

This manuscript describes both oral formulas and parenteral agents, e.g. MnSOD-liposomes which can reduce radiation exposure-induced biological effects. In addition, active immunization by non-toxic doses of radiation toxins, that we call the Specific Radiation Determinants (SRDs) can also be employed to reduce radiation toxicity. SRD immunization must be provided not less than 24 days before irradiation to have activity, and can be effective up to three years or more. Active immunization by radiation toxins can significantly improve the survival rate (up to 60 %) versus placebo-controlled irradiated animals. Our studies attempt to show the potential ability of specific antibodies to neutralize radiation toxins and thus substantially reduce the effects on

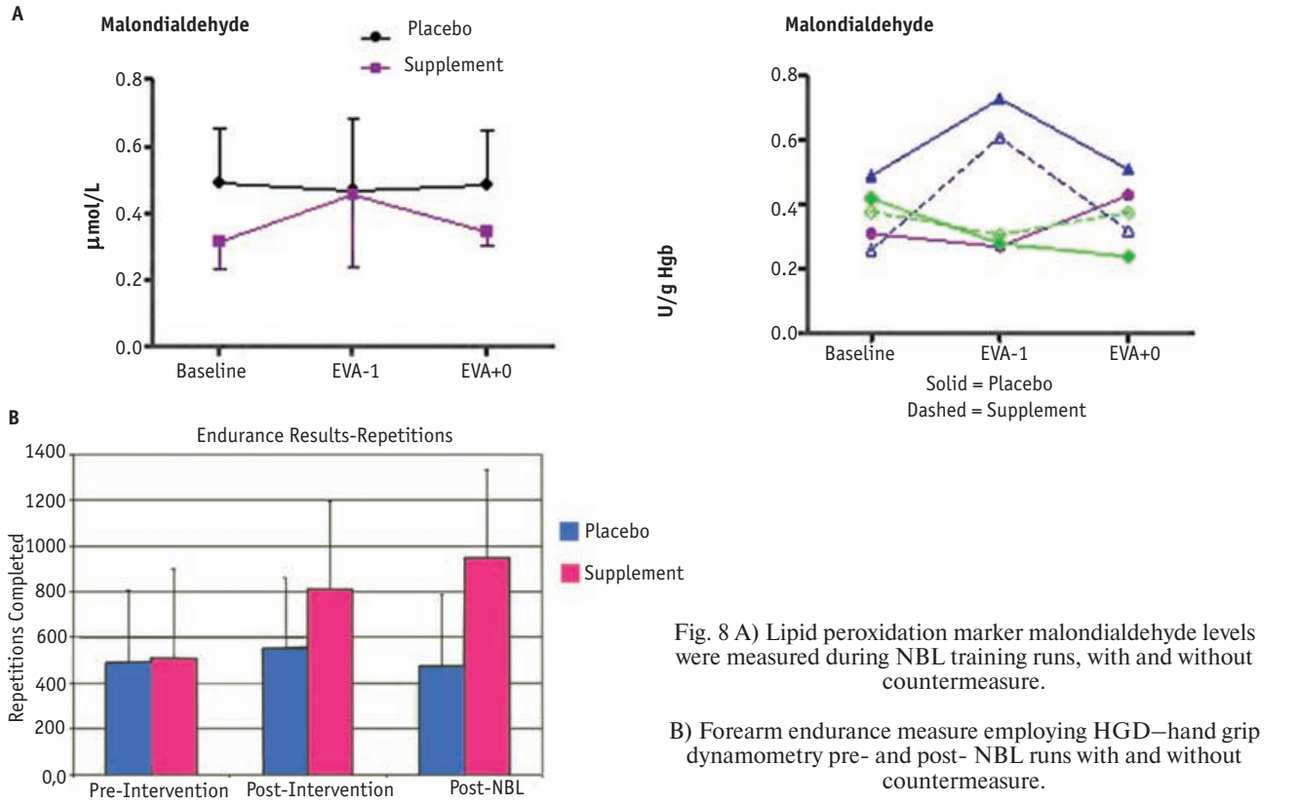

Fig. 8 A) Lipid peroxidation marker malondialdehyde levels were measured during NBL training runs, with and without countermeasure.

B) Forearm endurance measure employing HGD–hand grip dynamometry pre- and post- NBL runs with and without countermeasure.

radiation-induced neuro-, vascular, gastrointestinal, and hematopoietic toxicity. Antiradiation antibodies prevent the radiation-induced cytolysis of selected groups of cells that are sensitive to radiation. Anti-radiation antibodies derived from different phases of the ARS can compete with and thus prevent cytolysis mediated by cytotoxic lymphocytes. The therapeutic benefit of neutralization of SRD radiation toxins could make hemopoetic stem cell transplantation more effective. Antiradiation vaccine and IgG antibodies have shown activity in animals against several different types of radiation include gamma, heavy ions, and neutron irradiation [113, 114].

Conclusion

Developing countermeasures for radiation injury has a long and storied history, and is proving to be very challenging. Perhaps the era of high-dose single agents for this application is coming to an end. The authors of this manuscript feel that, in order to find a successful approach to protect the human against either acute or chronic sources of oxidative damage or radiation exposure, a multi pathway defense strategy must be developed. Oxidative damage in humans working or living in extreme environments is widespread and affects many cellular components. We have try to show that the downstream biological effects from this damage are variable, based on host factors, dose quality, magnitude and rate, as well as the presence or absence of countermeasures. Preliminary and pilot studies in vitro, in animal models and recently in humans, are showing some promise for both efficacy and safety/tolerability. The hope is, in these times of unpredictability in the operation of nuclear power facilities, possible terrorist weapons of mass destruction, and spaceflight operations, that this reported work has inspired the reader to bring forth new ideas and engage with authors in this important and meaningful pursuit.

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