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A Hypothesis on Biological Protection from Space Radiation Through the Use of New Therapeutic Gases

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Abstract. Radiation exposure to astronauts could be a significant obstacle for long duration manned space exploration because of current uncertainties regarding the extent of biological effects. Furthermore, concepts for protective shielding also pose a technically challenging issue due to the nature of cosmic radiation and current mass and power constraints with modern exploration technology. The concern regarding exposure to cosmic radiation is the biological damage it induces. As damage is associated with increased oxidative stress, it is important and would be enabling to mitigate and/or prevent oxidative stress prior to the development of clinical symptoms and disease. This paper hypothesizes a "systems biology" approach in which a combination of chemical and biological mitigation techniques are used conjunctively. It proposes using new, therapeutic, medical gases as both chemical radioprotectors for radical scavenging and biological signaling molecules for management of the body's response to exposure. From reviewing radiochemistry of water, biological effects of CO, H_2, NO, and H_2S gas, and mechanisms of radiation biology, it is concluded that this approach may have great therapeutic potential for radiation exposure. Furthermore, it also appears to have similar potential for curtailing the pathogenesis of other diseases in which oxidative stress has been implicated including, cardiovascular disease, cancer, chronic inflammatory disease, hypertension, ischemia/reperfusion injury, acute respiratory distress syndrome, parkinson's and alzheimer's disease, cataracts, and aging.

Keywords: space radiation, radiolysis, radiochemistry, applied electrochemistry, radiation shielding, therapeutic medical gas, reactive oxygen species, oxidative stress

THE CHALLENGE OF SPACE RADIATION

Galactic Cosmic Rays (GCR), solar energetic particles (SEP), and trapped energetic particles in a planetary magnetic field are natural sources for radiation in space. GCRs consist of highly energetic nuclei, predominately protons and He, but also with trace amounts of C, O, Ne, Si, Ca, and Fe ions. Particle energies can range from 100 MeV to 10 GeV per nucleon. Although the high charge and energy (HZE) nuclei are in trace amounts, they are still of concern and can cause more damage than the protons since they are more highly ionizing. Furthermore, GCRs and SEPs impinging on shielding material, atmosphere, or surface of a planet or satellite can produce secondary radiation, including energetic neutrons, from nuclear fragmentation of the primary ion and target atoms. This can introduce an additional component to the radiation field which makes shielding from HZE quite challenging and poses one of the principal unknowns in understanding the effects of HZE effects with human tissue (*Space Radiation Hazards…* 2006). Furthermore, while our bodies do possess a natural repair mechanism, radiation with a high linear energy transfer (LET) rate, like space radiation, is attributed to be more likely to cause double strand breaks in DNA that are relatively more difficult for our natural repair mechanisms to fix correctly (Chopping, et. al., 2002). While a week or month of this radiation at the dose rates naturally present likely will not have serious consequences, several year durations in space could.

The traditional paradigm for radiation protection is to minimize exposure time, maximize distance from radiation sources, and use shielding to attenuate and absorb radiation before it can deposit its energy in humans. In regards to minimizing exposure time, new propulsive technologies could reduce trip times but have yet to be developed and would not address the ability to remain at a location for long durations. It is impractical to maximize distance from radiation sources. In regards to shielding, aspects of attenuation by mass or deflection by magnetic fields or charge repulsion have been considered. Due to the phenomena of secondary radiation, shielding by other matter may require a significant amount of mass which could be impractical within current mass constraints in space systems. Due to the high energy of the space radiation, magnetic field and charge strengths required may be unreasonable due to current mass and power constraints in space systems along with other system design implications. In short, shielding space radiation is seemingly quite challenging. However, advances in biochemistry may reveal some more tools for radiation protection (Parker, 2006).

PARALLELS BETWEEN RADIATION CHEMISTRY OF WATER & RADIATION BIOLOGY

Radiolysis is the decomposition of water from exposure to ionizing radiation. Radiation chemistry of water has been well studied since the onset of nuclear power as water has been used for a coolant. Since mammalian cells are composed of about 80% water, it seemed natural that there exist similarities between radiation chemistry of water and radiation biology. It is these similarities from which analogues for radioprotective measures were inspired.

Chain of Events Initiated by Chemically Reactive Species

Radiolysis in nuclear systems causes a chain of events that ultimately manifest into systematic problems like corrosion and gas generation. Ionizing radiation creates chemically reactive radicals H_3O^+, e^-, H^+, H, and OH by ionizing and/or breaking the bonds of water molecules. These radicals then initiate a chain of chemical reactions within the water which can result in the formation of molecular decomposition products such as H_2, O_2, HO_2 and H_2O_2 (Lin, 1996). These oxidizing species alter the water composition and therefore electrochemical character which facilitates the manifestation of problems like corrosion or gas generation. As such, the nature in which systematic problems develop can be viewed as stemming from a chain of events that are initiated by ionization and propagated by a scheme of chemical reactions depending upon the net result of the ensuing chemistry.

This situation is similar in nature to a biological system and the pathogenesis of radiation related ailments. Ionization of key biological molecules can lead to chemical reactions which transform these molecules. This alters their biochemical function and can result in changes of cellular properties. This propagates from tissue to organ to system changes that ultimately manifest into clinical symptoms and ailments. Ionization of the molecules can occur both directly by radiation and indirectly by free radicals and reactive oxygen species (ROS) created by radiolysis. Free radicals and ROS like O_2^-, $^1O_2$, ·OH, ·OOH, NO· and H_2O_2 can cause cell injury or death by oxidative stress (Nakao, 2008) (Hanaoka, 2000). Oxidative stress can result in DNA damage, lipid peroxidation and also lead to a loss of protein after reductive remodeling of skeletal muscle due to undernutrition in space (Stein, 2002). Radiation-induced damage of chromosomes in lymphocytes may compromise the immune system's ability to prevent tumor development (Testard, et. al., 1996). Generally, the greatest risks from exposure are assumed to be cancer, cataracts, (Barr, et. al., 2007) and damage to the central nervous system (Koike, et. al., 2005).

Interestingly enough, oxidative stress has been implicated to play a role in the development of other diseases (Packer et al., 1997) (Sohal et al., 1996) including cardiovascular disease, cancer (Cerutti, et. al., 2002), chronic inflammatory disease (Ha., et. al., 2004), hypertension (Watson, et. al. 2008), ischemia/reperfusion injury (Nakao, 2008), acute respiratory distress syndrome (ARDS) (Tasaka, et. al., 2008), neurodegenerative diseases such as parkinson's disease and Alzheimer's disease (Nunomura, et. al., 2007) (Loh, et. al., 2006) and aging (Wei, et. al., 2001). Certain detrimental effects from space radiation on the dopaminergic system are similar to functional changes that occur from Parkinson's disease (Koike, 2005), diabetogenic problems associated with increased C-peptide excretion and insulin resistance (Tobin, 2002), as well as constipation due to malfunction of intestine. That is, the normal production and development of these disorders and diseases have also been associated with an increase of oxidative stress and inflammation similar to that which would likely be caused or increased by exposure to space radiation. Thus the nature of the problem seems similar to nuclear systems in that systematic

manifestations result from a chain of events initiated by ionization and propagated, in this case, by ensuing chemical reactions and biological responses.

Radical Scavenging & Antioxidants

The actual chemical reactions that ensue and their by-products depend upon what the radicals come into contact with. For example, in pure water, radical-radical interactions lead to the formation of the decomposition products while radical-decomposition product reactions lead to the reformation of water. In a nuclear system, manifestation of system level problems has been curtailed by interfering with the chain of events early on during the chemical stages through the use of additives that alter water composition. Whereas some additives have been found to promote and increase water decomposition, others have been found to suppress it (Schoenfeld, 2008). This occurs through scavenging in which the additives preferentially react with the radicals. Scavenging has the effect of removing reactive species from the system and thereby reduces their ability to participate in chemical reactions that cause decomposition. While there are various additives that preferentially react with decomposition products, the byproducts of the scavenging reaction are a factor as they are part of the composition. For example, ionic impurities react with the radicals but do so also at the expense of the water reformation process as these same radicals produce water from reacting with decomposition products.

$$OH + Br^- \rightarrow Br + OH^- \quad (1)$$

$$H + Br \rightarrow Br^- + H^+ \quad (2)$$

$$H + Cu^{++} \rightarrow Cu^+ + H^+ \quad (3)$$

$$OH + Cu^+ \rightarrow Cu^{++} + OH^- \quad (4)$$

However, the use of hydrogen as a scavenger produces water as a byproduct and thus promotes the water reformation process while neutralizing radicals.

$$H_2 + OH \rightarrow H_2O + H \quad (5)$$

$$H + H_2O_2 \rightarrow H_2O + OH \quad (6)$$

The ability of H_2 to suppress total oxidant concentrations in a water system exposed to radiation has long been recognized by the boiling water reactor (BWR) community and is referred to as hydrogen water chemistry (HWC). The addition of H_2 to water has the effect of suppressing the Open Circuit Potential (OCP) of the water (Figure 1) thereby electrochemically reducing the driving potential for corrosion.

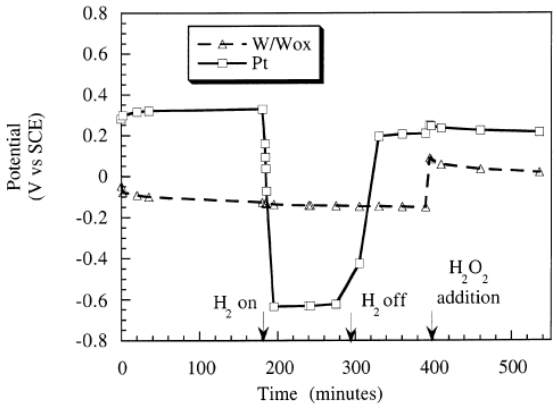

FIGURE 1. "OCP of the Tungsten/Tungsten-Oxide (ref. electrode) and Platinum Electrodes (vs. Saturated Calomel Electrode SCE) as a Function of Time. Plot Shows the Effect of Bubbling H_2 Gas Into Solution and the Addition of 0.1 M H_2O_2 on the OCP of These Electrodes" (Lillard, R.S. et al, 2000).

In a biological system, antioxidants have been seen to protect against oxidative stress and prevent the pathological process of a wide range of disease (Nakao, et al., 2009). The effect of antioxidants in reducing oxidative stress can be attributed to their ability to protect tissues from free radicals (Hanaoka, 2001) hinting towards a scavenging mechanism. Turner indicates, "A number of radiosensitizing chemicals and drugs are known. Some sensitize hypoxic cells, but have little or no effect on normally aerated cells. Other agents act as radioprotectors reducing biological effectiveness...which scavenge free radicals. Still other chemicals modifiers have little effect on cell killing but substantially enhance some multistep processes, such as oncogenic cell transformation" (Turner, 1995). Thus it appears that radical scavengers or antioxidants act as radioprotectors that chemically protect against indirect ionization by preferentially reacting with the reactive species thus reducing their ability to cause oxidative stress.

This dependency on scavenger type is similar to nuclear systems where the effect of the additive can either be to promote water decomposition or water reformation. One such example is the effect of oxygen. There appear to be parallels in the effect of oxygen to promote water decomposition in a nuclear system and increased radiosensitivity of cells in the presence of oxygen as shown in Figure 2C. Figure 2D also includes the effect of hydrogen and shows that when in excess of ROS like O_2 and H_2O_2, the water reformation process dominates as ROS are quickly scavenged. This begs the question of what the effect of H_2 would be on radiosensitivity. Also note worthy in figure 2 is the occurrence of an equilibrium where the amount of molecular decomposition byproducts from radiolysis remains constant. This reflects a balance between water decomposition and reformation process and hints at the ability for radical scavengers to effect which process dominates.

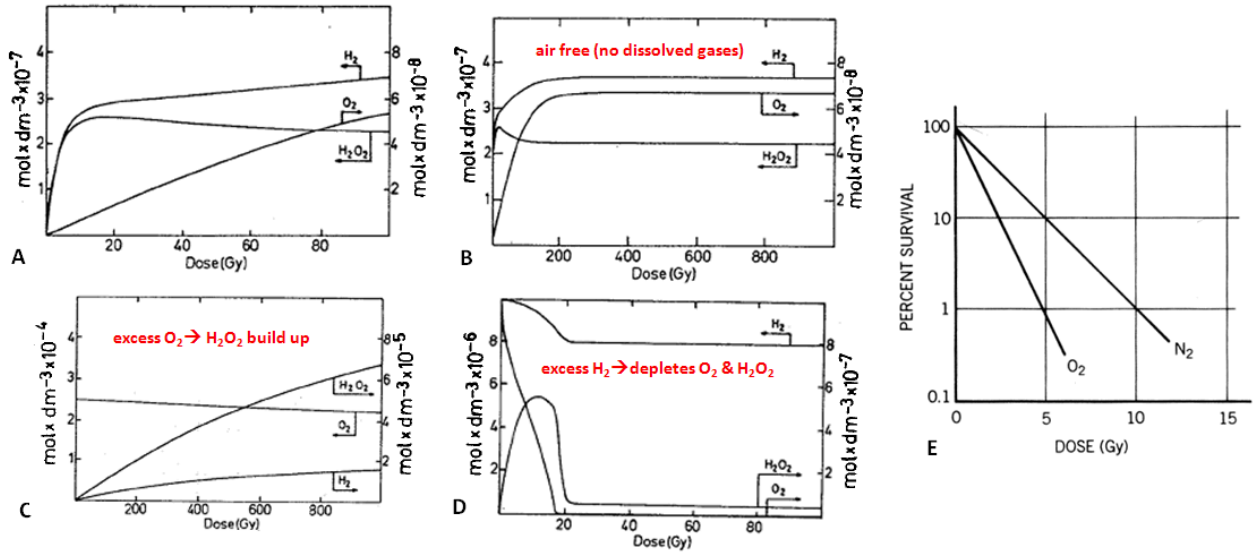

FIGURE 2. A-D (Bjergbakke et al., 1989) Reflect Water Decomposition by the Concentration of Radiolysis byproducts. B is an Extension of A and is Air Free Pure Water. Decomposition Ensues until H_2 in Excess of ROS. C is Effect of Dissolved O_2 in Excess of H_2 to Promote Decomposition. D is Effect of Dissolved Hydrogen in Excess of O_2 to Scavenge. E (Turner, 1995) Shows Effect of O_2 as a Biological Radiosensitizer. N_2 is Also Shown Which Begs the Question of the Effect of H_2.

The Net Effect of Competing Processes & Natural Repair Mechanisms

Radiolysis of water results in chemical reactions that are part of two competing processes of decomposition and reformation. Decomposition will still occur even in the presence of additives but they serve to affect the chemical reactions such that one process becomes more dominate and thus the altering the net effect. This was seen somewhat in figure 2 and is shown more explicitly in figure 3 which shows that the threshold for which negative effects can manifest can be increased through bolstering the scavenging capacity and altering the balance so that the favorable processes is dominant.

As mentioned earlier, free radicals and ROS were identified as the root cause of oxidative stress in a biological system. While these species can be derived from exposure to external sources like X-rays, ozone, cigarette smoke,

air pollutants and industrial chemicals (Dean, 1997), they also are naturally generated during a variety of energy-generating biochemical reactions and cellular functions (Nakao, 2008). Furthermore, the ROS actually serve a necessary function as signaling molecules that critically modulate the activation of the immune system and thus participate in antibacterial defense (Reth, 2002).

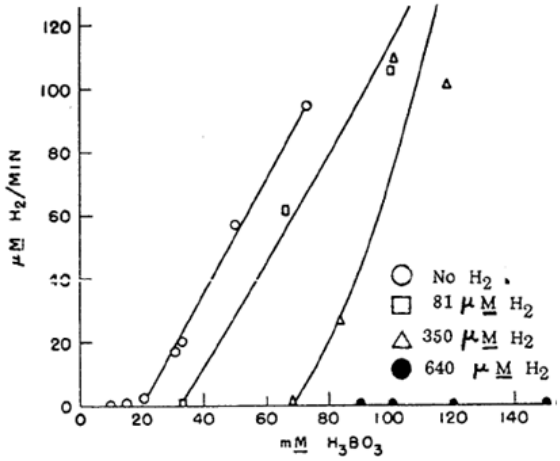

FIGURE 3. A (Hart, 1956) Relative Contribution of Water Decomposition Process Associated with Boric Acid Concentrations. Relative Contribution of Water Reformation Process Associated with Initial Amount of Dissolved H_2. Manifestation of Negative Systematic Effects Reflected by H_2 Gas Generation Rates from Water Radiolysis. The Addition of dissolved H_2 Increases the Scavenging Capacity of the Water Therefore Increasing the Threshold and Delaying the Onset of when Decomposition Becomes the Dominant Process.

Oxidative stress occurs when there is an imbalance between antioxidants and ROS and free radicals (Halliwell, et. al., 1992) such as when radiation exposure increases the amount of these species through production by ionization. Thus, in the biological system, it similarly appears to be a situation of competing processes between biochemical damage and repair processes. Chopping notes, "The cell is protected by different DNA repair mechanisms which try to restore the damage. We don't know the details, except when the repair goes wrong (e.g. a replacement of a lost nucleotide by a 'wrong" base pair, etc.)… The cell contains natural radical scavengers. As long as they are in excess of the radiolysis products, the DNA may be protected. When the products exceed the amount of scavengers, radiation damage and cancer induction may occur. In principle, there could thus be a threshold dose for radiation damage, at which the free radicals formed exceed the capacity of scavenging. The scavenging capacity may differ from individual to individual depending on his/her physical condition" (Chopping et al., 2002). Experimental investigations regarding long-duration space flights in particular clearly showed increased oxidative stress markers and a reduction in antioxidants after these flights (Hollander, 1998) (Stein, 2002). Kennedy et al. demonstarted that exposure to space radiation may compromise the capacity of the host antioxidant defense system and that this adverse biological effect can be prevented, at least partially, by dietary supplementation with agents expected to have effects on antioxidant activities (Kennedy, 2007). Interestingly and similarly so, the radiation resistance of the bacteria *Deinococcus radiodurans* that can grow under chronic γ radiation (50 Gy/hr) or recover from acute doses greater than 10 kGy has been attributed to the role of antioxidants in mitigating the extent of oxidative damage (Daly, 2004) (Ghosal, 2004) (Daly, 2007). Thus there appear to be similarities between the nuclear and biological systems in how use of scavengers can enhance and bolster the favorable process thereby increasing the natural radiation resistance of the system.

SYSTEMS BIOLOGY: BIOCHEMICAL CONJUNCTIVE APPROACH

Over the course of the last century, a wealth of knowledge has been accumulated on the effect of radiation on biological systems. Areas spanning in scope from DNA damage up to changes in physiology have received extensive study. To date, biology studies of radiation damage have largely focused on components of DNA repair systems such ataxia telangiectasia mutated gene (ATM). More recently, however, it has been found that modification of key molecular targets can protect tissue from radiation induced fibrosis (25Gy) (Isenberg et al.,

2008) (Maxhimer et al., 2009). It has also been found that changes in APOE (Apolipoprotein E) genotype dramatically influences survival following Total Body Irradiation (TBI) in murine models. These results imply that modification of key molecular targets to induce biological changes in the host can protect tissue from radiation damage. Thus, a strategy of (1) interrupting the chain of events and (2) bolstering repair processes and reducing damage processes could have a great effect on increasing the threshold after which radiation damage propagates to the systematic symptoms. We suggest the use of new therapeutic medical gases to administer radioprotectors and biological signaling molecules to work conjunctively in preventing, protecting, and repairing radiation damage. Turner notes that, "for carcinogensis or transformation, for example, such biological promoters (radioprotectors) can dwarf the effects of physical factors, such as LET and dose rate, on dose-response relationships" (Turner, 1995).

Radioprotectors have been implicated to work by the following chemical and biological mechanisms:

1. radical scavenging of toxic decomposition products of free radicals and ROS
2. repair of biological molecules by donation of H atoms since hydrogen bonds are among the weakest in biological molecules and such are the first to be broken (Casarett, 1968)
3. interaction with cellular components (binding, altering metabolic pathway, etc.)

Interaction with cellular components can have biological effects that lend to radioprotection like hypoxia, alteration of metabolic state, and anti-apoptotic properties. Tissue hypoxia decreases the radiosensitivity of cells and can be produced chemically by impairing oxygen transport (binding up hemoglobin with another molecule) or biologically by restricting blood flow (vasoconstrictor drug) or lowering blood pressure (vasodilator drug). Inducing a hypometabolic state which resembles hibernation, may contribute to tolerance against oxidative stress. Metabolic rates in hibernating marmots and ground squirrels help delay the onset of obvious damage. Also, survival times for guinea pigs that have received massive doses of radiation (>6000 rads) have been extended from several hours to about 4 days through the use of central nervous system depressants (pentobarbital) where it has been attributed to partial protection from central nervous system syndrome (Casarett, 1968). Furthermore, a hypometabolic status may also prove to be an ideal therapy for various shock or trauma states in which dramatic reduction in metabolic demands may be highly protective (Lefer, 2007). Anti-apoptotic properties can mitigate organ damage such as in IR injury by reducing the amount of cellular self destruction. Interference with mitosis and DNA synthesis can slow cells in their radio-resistant phase of cell division and afford more time for natural repair of the cell prior to replication of the damage.

Therapeutic Medical Gases

NO, CO, H_2S and H_2 are gaseous signaling molecules in humans. These molecules act as transmitters of information between cells by chemical interacting with cell receptors to trigger a response within the cell. These comprise some of the medical gases of interest and many of them act both on the chemical level in the form of antioxidant radical scavenging and on the biological level in the form anti-inflammatory, anti-apoptotic, and other biological effects. Extensive and more detailed information about these gases in a therapeutic role can be found in Nakao et al. (2009) which provides a detailed description of medical gases of interest and their properties and Huang (2010) which provides detailed information pertaining in particular to H_2.

Hydrogen

Hydrogen has only recently been considered for therapeutic applications for radiation exposure (Liu, 2009) (Schoenfeld, M.P. et al., 2010) but recent results have demonstrated its radioprotective effects in cultured cells and mice (Qian, 2010). Hydrogen properties as a medical gas are summarized in Table 1. Hydrogen may have potential as a safe and potent therapeutic medical gas, as well as several potential advantages over current pharmacological therapies for the following reasons:

- It is highly diffusible and as such could potentially reach subcellular compartments, such as mitochondria and nuclei, which are the primary site of ROS generation and DNA damage (Ohsawa, 2007) and are also notoriously difficult to target pharmacologically.

- Its hyporeactivity with other gases at therapeutic concentrations may allow hydrogen to be administered with other therapeutic gases, including inhaled anaesthesia agents (Nakao, 2010).
- H_2 may spare the innate immune system while still allowing phagocytosis of infecting organisms. When tested *in vitro*, it did not eliminate O_2^- or H_2O_2 which have important functions in neutrophils and macrophages as they must generate ROS in order to kill some types of bacteria engulfed by phagocytosis (Oshawa, 2007). It is not clear whether a similar reaction preferentially occurs under complex biological conditions. Experimental studies have demonstrated that hydrogen has potent therapeutic efficacies on both parasite infection (Gharib, 2001) and polymicrobial sepsis (Xie, 2010).
- No adverse effects have been found in humans drinking hydrogen water in a study that examined the effects of drinking hydrogen-rich water (HW) for radiation-induced late adverse effects (Kajiyama, 2009) (Nakao, 2010). Studies showed that the consumption of HW for 6 months resulted in significant decrease of serum level of derivatives of Reactive Oxidative Metabolites (dROMs) and increase of biological antioxidant power determined by Free Radical Analytical System (FRAS). No severe adverse effects were seen during follow up period. These results suggest that drinking HW improved Quality of Life (QOL) in patients with radiotherapy associated with decrease of oxidative injury markers.

TABLE 1. Cited Properties of H_2 as a Medical Gas with Suggested Chemical/Biological Mechanisms.

Biochemical Mechanism	Notes
radical scavenging antioxidant	• selectively reduces hydroxyl radicals (•OH) and peroxynitrite ($ONOO^-$) but did not eliminate O_2^- or H_2O_2 when tested in *in vitro* (Ohsawa, 2007) • does not decrease the steady-state levels of nitric oxide (NO) (Ohsawa, 2007) which may be beneficial as endogenous NO signaling pathways modulate pulmonary vascular tone and leukocyte/endothelial interactions (Pinsky, 1994) • increases antioxidant enzymes such as catalase, superoxide dismutase or heme oxygenase-1 (Kajiyama, 2008) (Xie et al., 2010)
anti-apoptotic	• postulated to inhibit caspase-3 activation (Sun, 2009)
anti-inflammatory	• down-regulation of pro-inflammatory cytokines, such as interleukin (IL)-1 β, IL-6, chemokine (CC motif) ligand 2 and tumor necrosis factor-α (TNF-α) (Mao, 2009) (Chen, 2004)

Nitric Oxide

TABLE 2. Cited Properties of NO as a Medical Gas with Suggested Chemical/Biological Mechanisms.

Biochemical Mechanism	Notes
radical scavenging antioxidant	• NO reacts with peroxy and oxy radicals generated during the process of lipid peroxidation. The reactions between NO and these ROS can terminate lipid peroxidation and protect tissues from ROS-induced injuries (Padmaja, 1993) • induces the rate-limiting antioxidant enzyme, heme oxygenase (HO)-1 thus imparting resistance to H_2O_2 induced cell death (Kim, 1995) • in bacteria, activates the redox-sentive transcriptional regulator protein (oxyR), resulting in the subsequent expression of protein protective against ROS (Nunoshiba et al., 1993)
anti-inflammatory	• inhibiting P-selectin expression and leukocyte recruitment (Ahluwalia, 2004)
decreased radiosensitivity	• vasodilator through relaxation of vascular tone by stimulating soluble guanylate cyclase (sGC) and increased cGMP content in vascular smooth muscle cells (Nakao, et al., 2009)

NO medical properties are summarized in Table 2 and effects are shown in Figure 4. NO regulates platelet activity, preservation of the normal structure of the vessel wall and causes blood vessel dilation which may increase tissue blood supply (Nakao, et al., 2009). This could abate inflammatory response and thus protect tissue from oxidative injury. It also may enhance the natural repair as mechanism as it is believed to be more effective in a living organism, where the cells are in continuous exchange with the surrounding cells and body fluids, than in the tissue samples often studied in the laboratory (Chopping et al., 2002). Results from NO studies that have examined the ability of patients to inhale NO to improve outcome of acute respiratory distress syndrome (ARDS) have had discrepant results from positive, negative or neutral outcomes. Thus NO may be linked with both protective and

toxic effects depending upon concentrations, source, timing of administration and the environment suggesting a narrow window for administration in the treatment of oxidative injuries (Bolli, 2001). Reduction of excessive and deleterious NO effects appear to be controlled by blocking NO/cGMP signaling through thrombospondin-1 signaling via its receptor CD47. This has shown to both maintain the viability of normal tissues against radiation induced fibrosis in murine models following total body irradiation (25 Gy) and increase the radiosensitivity of tumors (Isenberg, 2008) (Isenberg 2009) (Maxhimer, 2009).

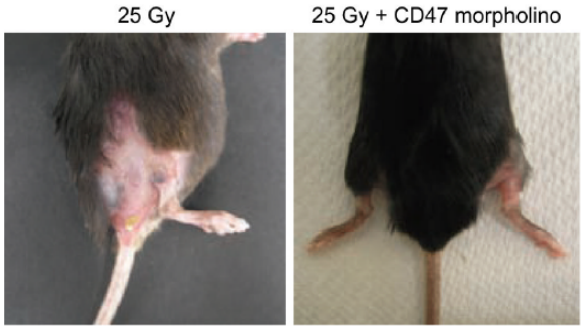

FIGURE 4. "After 8 weeks, a representative image shows signs of fibrotic contractures only in the untreated irradiate hind limb" (Maxhimer, 2009).

Carbon Monoxide

TABLE 3. Cited Properties of CO as a Medical Gas with Suggested Chemical/Biological Mechanisms.

Biochemical Mechanism	Notes
radical scavenging antioxidant	• binds to the heme moiety of mitochondrial cytochrom *c* oxidase. By binding to the heme, CO may prevent degradation of heme proteins which induce tissue injury by rapidly promoting peroxidation of the lipid membranes of cells (Nath, 1995) (Kumar, 2005) • reduces mitochondria-derived ROS thus resulting in lower levels of ROS generation in which an adaptive cellular response is triggered leading to cell survival rather than cell death (Bilban et al, 2006) (Taille, et al, 2005) (Zuckerbraun et al, 2007) • can induce HO-1 in cells to protect against injury (Lee, 2006) (Sawle, 2005) (Hegazi, 2005). Thus, detrimental excess of heme can be immediately removed by HO-1 enzymatic activity induced by CO
decrease radiosensitivity	• impedes O_2 transport as it binds to hemoglobin with an affinity 240 times higher than that of O_2

Table 3 summarizes medical properties of CO gas. While the adverse effects of inhaled CO are a major concern for clinical use, experimental models have demonstrated that potent therapeutic efficacies exist at low concentrations (Nakao, et al., 2009) (Han, 2009). Soluble forms of CO, such as CO-releasing molecules, may overcome the problem of tissue hypoxia and allow clinical application (Motterlini, 2005) (Nakao et al., 2006). Recent animal studies have shown discrepant results between exhibiting and not exhibiting anti-inflammatory effects (Nakao, et al., 2009). These discrepancies may be attributed to species specific differences in the affinity of CO for hemoglobin, or physiological differences such as respiratory rate and sensitivity to lipopolysaccharides (endotoxins) (Redl, 1993) (Klimisch, 1975). Figure 5 shows that administration of H_2/CO mixtures has been shown to reduce structural damage to hearts in Lewis rats undergoing heart transplantation (HTx) in which oxidative stress injury is caused by ischemia/reperfusion (Nakao, et al., 2009) rather than radiation exposure.

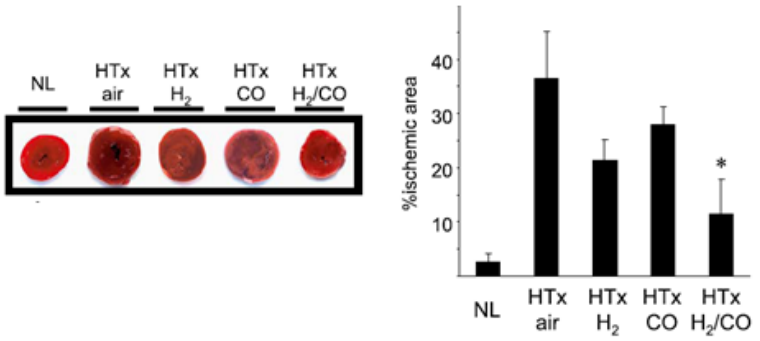

FIGURE 5. Extent of Gross Structural Damage to Heart Graft Was Evaluated by TTC staining 3 h After Reperfusion. H_2 and CO Inhalation Reduced Ischemic Area Following Hear Grafts but with Only Slight Significance. Significant Reduction is Seen by Dual Treatment (Nakao, et al., 2009).

Hydrogen Sulfide

H_2S is produced enzymatically at micromolar levels in mammals and is believed to help regulate body temperature and metabolic activity at physiological concentrations (Kamoun, 2004) (Lowicka et al., 2007). It has been implicated as the mechanism by which consumption of garlic attenuated cardiovascular disease where production of the gas has been demonstrated to occur by bioconversion of garlic-derived polysulfides by red blood cells (Lowicka et al. 2007). According to Lefer (2007), it appears that H_2S possesses all of the positive effects of NO with the capacity to from the toxic metabolite such as $ONOO^-$. The medical properties of H_2S are summarized in Table 4.

TABLE 4. Cited Properties of H_2S as a Medical Gas with Suggested Chemical/Biological Mechanisms.

Biochemical Mechanism	Notes
radical scavenging antioxidant	• antioxidant inhibitor of peroxynitrite-mediated processes via activation of N-methly-D-aspartate (NMDA) receptors (Whiteman et al., 2004) • shield cultured neurons from oxidative damage by increasing levels of glutathione (Kimura et al., 2004) • induce upregulation of HO-1, anti-inflammatory and cytoprotective genes (Oh et al., 2006) (Qingyou, 2004) • inhibits myeloperoxidase and destroys H_2O_2 (Laggner, et al., 2007)
anti-apoptotic	• reduces IR induced apoptosis via reduction of cleaved caspase-3 and cleaved poly (ADP-ribose) polymerase (PARP) (Sodha, 2008)
anti-inflammatory	• inhibit leukocyte adherence in the rat mesenteric microcirculation during vascular inflammation (Lefer, 2007)
decrease radiosensitivity	• vascorelaxtion and vasodilation of isolated blood vessels via vascular smooth muscle K_{ATP} channel-mediated hyperpolarization (Lefer, 2007) (Nakao, et al., 2009) • transiently and reversibly inhibiting mitochondrial respiration (Lefer, 2007)
metabolic alteration	• produces a "suspended animation-like" metabolic status with hypothermia and reduced oxygen demand in pigs (who received it intravenously) (Simon, 2008) and mice (who received hydrogen sulphide via inhalation) (Blackstone, 2005) (Blackstone, 2007).

CONCLUSION

We hypothesize a systems approach of chemical radioprotectors in conjunction with biological signaling molecules could disrupt the chain of events initiated by radiation exposure and interfere with pathogenesis. This could have a profound positive effect on survival as it addresses prevention, protection, and repair post damage. In particular, hydrogen or combinations of other medical gases could be administered to astronauts by either inhalation of non-flammable gas mixture as part of the spacecraft or suit atmosphere or drinking gas rich water. This represents a novel and feasible preventative/therapeutic strategy to address radiation-induced adverse events and thus the

challenge of space radiation. While more studies are warranted to apply this therapy for space travel and determine details of optimum gas mixtures and therapy administration plans, it appears that it represents a potentially novel therapeutic and preventative strategy that may also ameliorate symptoms for other oxidative stress related diseases as has been shown in relevant ground-based (animal) models.

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REFERENCES

Abraini, J.H. et al., "Psychophysiological Reactions in Humans During an Open Sea Dive to 500 m with a Hydrogen-Helium-Oxygen mixture," *J. Appl. Physiol.*, 76, 1994. pp. 1113–1118.

Ahluwalia, A. et al., "Antiinflammatory activity of soluble guanylate cyclase: cGMP-dependent down-regulation of P-selectin expression and leukocyte recruitment," *Proc. Natl. Acad. Sci.* U. S. A., 101, 2004, pp. 1386–13891.

Barr Y.R., Bacal K, Jones, J.A., Hamilton, D.R., "Breast Cancer and Spaceflight: Risk and Management," *Aviat Space Environ Med*, 78, 2007, pp. A26–37.

Bilban, M. et al., "Carbon monoxide orchestrates a protective response through PPARgamma," *Immunity*, 24, 2006, pp. 601–610.

Bjergbakke, E., Draganic, Z. D., Sehested, K., and Draganic, I.G., "Radiolytic Products in Waters Part I: Computer Simulation of Some Radiolytic Processes in the Laboratory," in *Radioehimiea* Acta, vol. 48, Munehen, 1989, pp. 65-71.

Blackstone, E. et al., "H_2S induces a suspended animation-like state in mice," *Science*, 308, 518, 2005.

Blackstone, E., et al., "Suspended animation-like state protects mice from lethal hypoxia," *Shock*, 27, 2007, pp. 370–372.

Bolli, R., "Cardioprotective function of inducible nitric oxide synthase and role of nitric oxide in myocardial ischemia and preconditioning: an overview of a decade of research," *J. Mol. Cell Cardiol.*, 33, 2001, pp. 1897–1918.

Buchholz, B.M. et al., "Hydrogen inhalation ameliorates oxidative stress in transplantation induced 170 intestinal graft injury," *Am J Transplant*, 8, 2008 pp. 2015–24

Casarett, A.P., *Radiation Biology*, Prentice-Hall, Inc., New Jersey, 1968, pp. 249-262Chopping, G., Liljenzin, J., Rydberg, J., *Radiochemistry and Nuclear Chemistry*, Butterworth-Heinemann, 3rd. ed., 2002.

Cerutti, P.A. and Trump, B.F., "Inflammation and oxidative stress in carcinogenesis," *Cancer Cells*, 3, 1991, pp. 1-7.

Chen X.L. et al., "Superoxide, H_2O_2, and iron are required for TNF-alpha-induced MCP-1gene expression in endothelial cells: role of Rac1 and NADPH oxidase," *Am J Physiol Heart Circ Physiol*, 286, 2004, pp. 1001 – 1007.

Chopping, G., Liljenzin, J., Rydberg, J., "*Radiochemistry and Nuclear Chemistry*", Butterworth-Heinemann, 3rd. ed., 2002.

Dean, R.T., "Biochemistry and Pathology of Radical-Mediated Protein Oxidation," *Biochem. J.,* **324**, 1997, pp. 1-18.

Daly, M..J., et. al., "Accumulation of Mn(II) in *Deinococcus radiodurans* Facilitates Gamma Radiation Resistance," *Scienceexpress*, 30 September 2004.

Daly, M. J., et. al., "Protein Oxidation Implicated as the Primary Determinant of Bacterial Radioresistance," *PLoS Biol.*, 5(4), April 2007.

Fujita, K et al, "Hydrogen 172 in drinking water reduces dopaminergic neuronal loss in the 1-methyl-4- 173 phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease," *PLoS One*, 4, 2009.

Ha, H., Park, J., Kim, Y.S., and Endou, H., "Oxidative stress and chronic allograft nephropathy, *Yonsei. Med. J.*, 45, 2004, pp. 1049–1052.

Halliwell, B., Gutteridge, J.M., and Cross, C.E., "Free Radicals, Antioxidants, and Human Disease: Where are we Now?," *J. Lab. Clin. Med.,* **119**, 598-620, 1992.

Hanaoka, K., "Antioxidant Effects of Water Produced by Electrolysis of Sodium Chloride Solutions," *Journal of Applied Electrochemistry,* **31**, pp. 1307-1313.

Han, W., Lijun, W. Shaopeng, C. and Yu, K.N., "Exogenous Carbon Monoxide Protects the Bystander Chinese Hamster Ovary Cells in Mixed Coculture System After Alpha-Particle Irradiation," *Carcinogenesis*, vol. 31 no. 2, 2010, pp. 275-280

Hart, E.J., McDonell, W.R., and Gordon, S., "The Decomposition of Light and Heavy Water Boric Acid Solutions by Nuclear Reactor Radiations," in proceedings of *International Conference on the Peaceful Uses of Atomic Energy*, Geneva 1955, P/839, Vol. 7, United Nations, New York, 1956, pp. 597.Hegazi, R.A. et al., "Carbon monoxide ameliorates chronic murine colitis through a heme oxygenase 1-dependent pathway," *J. Exp. Med.*, 202, 2005, pp. 1703–1713.

Hollander J, Gore M, Fiebig R, Mazzeo R, Ohishi S, Ohno H, et al., "Spaceflight downregulates antioxidant defense systems in rat liver," *Free Radic Biol Med*, **24**, 1998, pp. 385–90.

Huang, C., Kawamura, T., Toyoda, Y., Nakao, A., "Recent Advances in Hydrogen Research as a Therapeutic Medical Gas," *Free Radical Research*, **44**(9), September 2010, pp. 971-982.

Isenberg et al., "Thrombospondin-1 and CD47 Limit Cell and Tissue Survival of Radiation Injury," *Am. J. Pathol.*, **173**(4), October 2008, pp. 1100-12.

Gharib B. et al., "Anti-inflammatory properties of molecular hydrogen: investigation on parasite-induced liver inflammation," *C R Acad Sci*, III, 2001 pp. 324:719 – 724.

Ghosal, D., et. al., "How radiation Kills Cells: Survival of *Deinococcus radiodurans* and *Shewanella oneidenis* under Oxidative Stress," *FEMS Microbiology Reviews* 2005.

Kajiyama, S, et al, "Supplementation of hydrogen-rich water improves lipid and glucose metabolism in patients with type 2 diabetes or impaired glucose tolerance," *Nutr Res*, 28, 2008, pp. 137-143.

Kamoun, P, "Endogenous Production of Hydrogen Sulfide in Mammals," *Amino. Acids.*, **26**, 2004, pp. 243-254.

Koike Y, Frey MA, Sahiar F, Dodge R, Mohler S., "Effects of HZE Particle on the Nigrostriatal Dopaminergic System in a Future Mars Mission," *Acta Astronaut*, 56, 2005, pp. 367–78.

Kennedy, A..R, Guan, J., Ware, J.H., "countermeasures against space radiation induced Oxidative Stress in Mice," *Radiat Environ Biophys*, **46**, 2007, pp. 201-203.

Kim, Y.M., Bergonia, H., and Lancaster, J.R., Jr., "Nitrogen oxide-induced autoprotection in isolated rat hepatocytes," *FEBS. Lett.*, 374, 1995, pp. 228–232.

Kimura, Y. and Kimura, H., "Hydrogen Sulfide Protects Neurons from Oxidative Stress," *FASEB J.*, **18**, 2004, pp. 1165-1167.

Klimisch, H.J., Chevalier, H.J., Harke, H.P., and Dontenwill, W., "Uptake of carbon monoxide in blood of miniture pigs and other mammals," *Toxicology*, 3, 1975, pp. 301–310.

Kumar, S. and Bandyopadhyay, U, "Free heme toxicity and its detoxification systems in human," *Toxicol. Lett.*, 2005, pp. 175–188.

Lowicka, E. and Beltowski, J, "Hydrogen Sulfide (H_2S)—the Third Gas of Interest for Pharmacologist," *Pharmacol. Rep.*, **59**, 2007, pp. 4-24.

Lee, B.S. et al., "Carbon monoxide mediates heme oxygenase 1 induction via Nrf2 activation in hepatoma cells," *Biochem. Biophys. Res. Commun.,* 343, 2006, pp. 965–972.

Lillard, R.S., Pile, D.L., Butt, D.P., "The Corrosion of Materials in Water Irradiated by 800 MeV Protons," *Journal of Nuclear Materials*, **278**, 200, pp. 277-289.

Lin, C. C., *Radiochemistry in Nuclear Power Reactors*, Committee on Nuclear and Radiochemistry, National Research Council, National Academy Press, Washington, D.C., 1996, pp. 125-142.

Liu, C., et al. "Hydrogen Therapy may be an effective and specific Novel Treatment for Acute Radiation Syndrome," *Medical Hypotheses*, 2009.

Loh, K.P., Huang, S.H., De Silva, R., Tan, B.K., and Zhu, Y.Z., "Oxidative stress: apoptosis in neuronal injury," *Curr. Alzheimer. Res.*, 3, 2006, pp. 327–337.

Mao, Y.F. et al., "Hydrogen-rich saline reduces lung injury induced by intestinal ischemia/reperfusion in rats," *Biochem Biophys Res Commun,* 381, 2009, pp. 602 – 605.

Maxhimer et al., "Radioprotection in Normal Tissue and Delayed Tumor Growth by Blockade of CD47 Signaling," *Sci Transl Med.*, **1**(3), October 21, 2009, 3ra7.

Motterlini, R., Mann, B.E., and Foresti, R., "Therapeutic applications of carbon monoxide-releasing molecules," *Expert Opin Investig. Drugs*, **14**, 2005, pp. 1305–1318.

Nakao, A. et al., "Ex vivo application of carbon monoxide in university of wisconsin solution to prevent intestinal cold ischemia/reperfusion injury," *Am. J. Transplant.*, **6**, 2006, pp. 2243–2255.

Nakao, A., Kaczorowski, D.J., Sugimoto, R., Billiar, T.R., and McCurry, K.R., "Application of heme oxygenase-1, carbon monoxide and biliverdin for the prevention of intestinal ischemia/reperfusion injury," *J. Clin. Biochem. Nutr.*, 42, 2008, pp. 78–88.

Nakao, A., Sugimoto, R., Billiar, T.R., McCurry, K. R., "Therapeutic Antioxidant Medical Gas," *J. Clin. Biochem. Nutr.,* **44**, January 2009, pp. 1-13.

Nakao, A., Toyoda, Y., Sharma, P., Evans, M. and Guthrie, N., "Effectiveness of Hydrogen Rich Water on Antioxidant Status on Subjects with Potential Metabolic Syndrome—An Open Label Pilot Study," *J. Clin. Biochem. Nutr.*, **46**, March 2010, pp. 140-149.

Nakao, A. et al., "Amelioration of rat cardiac cold ischemia/reperfusion injury with inhaled hydrogen or carbon monoxide, or both," *J Heart Lung Transplant*, 29, 2010, pp. 544–553.

Nath, K.A. et al, "Heme protein-mediated renal injury: a protective role for 21-aminosteroids in vitro and in vivo," *Kidney Int.*, 47, 1995, pp. 592–602

Nunomura, A., Moreira, P.I., Takeda, A., Smith, M.A., and Perry, G., "Oxidative RNA damage and neurodegeneration," *Curr. Med. Chem.*, 14, 2007, pp. 2968–2975.

Nunoshiba, T., et al., "Activation by nitric oxide of an oxidative-stress response that defends Escherichia coli against activated macrophages," *Proc. Natl. Acad. Sci.*, U. S. A., **90**, 1993, pp. 9993–9997.

Oh, G.S., et al., "Hydrogen Sulfide Inhibits Nitric Oxide Production and Nuclear Factor-kappaB via heme oxygenase-1 Expression in RAW264.7 Macrophages Stimulated 2with Lipopolysaccharide," *Free Radic. Biol. Med.,* **41**, 2006, pp. 106-119.

Ohsawa I et al., "Hydrogen Acts as a Therapeutic Antioxidant by Selectively Reducing Cytotoxic Oxygen Radicals," *Nat Med*, 13, 2007 pp. 688-694.

Packer, L. and Fuchs, J.J., 'Vitamin C in Health and Disease' (Marcel Dekker, New York, 1997). 6. R.S. Sohal and R. Weindurch, Science 273 (1996) 59.

Padmaja, S. and Huie, R.E., "The reaction of nitric oxide with organic peroxyl radicals," *Biochem. Biophys. Res. Commun.,* 195, 1993, pp. 539–544.

Parker, E. N., "Shielding Space Travelers," *Scientific American*, March 2006, pp. 40-47.

Pinsky, D.J. et al., "The nitric oxide/cyclic GMP pathway in organ transplantation: critical role in successful lung preservation," *Proc Natl Acad Sci*, 91, USA 1994, pp. 12086 – 12090.

Qian, L. et al., "Radioprotective effect of Hydrogen in Cultured Cells and Mice," *Free Radical Research*, **44(3)**, March 2010, pp. 275-282.

Qingyou, Z., et al., "Impact of Hydrogen Sulfide on Carbon Monoxide/Heme Oxygenase Pathway in the Pathogenesis of Hypoxic Pulmonary Hypertension," *Biochem. Biophys. Res. Commun.*, **371**, 2004, pp. 30-37.

Redl, H., Bahrami, S., Schlag, G., and Traber, DL, "Clinical detection of LPS and animal models of endotoxemia," *Immunobiology*, 187, 1993, pp. 330–345.

Reth, M., "Hydrogen peroxide as second messenger in lymphocyte activation," *Nat. Immunol.*, 3, 2002, pp. 1129–1134.

Sawle, P. et al., "Carbon monoxide-releasing molecules (CO-RMs) attenuate the inflammatory response elicited by lipopolysaccharide in RAW264.7 murine macrophages," *Br. J. Pharmacol.*, 145, 2005, pp. 800–810.

Schoenfeld, M. P., "A Review of Radiolysis Concerns for Water Shielding in Fission Surface Power Applications," *Space Technology and Applications International Forum (STAIF 2008)*, AIP Conference Proceedings 969, New York, 2008, pp. 337-347.

Schoenfeld, M.P., et al., "Hydrogen therapy may reduce the risks related to radiation-induced oxidative stress in space flight," *Med Hypotheses,* 2010.

Simon, F. et al., "Hemodynamic and Metabolic Effects of Hydrogen Sulfide During Porcine Ischemia/Reperfusion Injury," *Shock*, In press, 2008.

Sodha, N.R., et al., "The Effects of Therapeutic Sulfide on Myocardial Apoptosis in Response to Ischemia-Reperfusion njury," *Eur. J. Cardiothorac. Surg.*, **33**, 2008, pp. 906-913.

Sohal, R.S., Weindurch, R., *Science*, **273**, 1996, pp. 59.

Space Radiation Hazards and the Vision for Space Exploration, Ad Hoc Committee on the Solar System Radiation Environment and NASA's Vision for Space Exploration: A Workshop Space Studies Board Division on Engineering and Physical Sciences, The National Academies Press, Washington, D.C., 2006. pp. 7-37.

Stein T.P., "Space Flight and Oxidative Stress," *Nutrition*, 18, 2002, pp. 867–71.

Strocchi A, Levitt, M.D., "Maintaining intestinal H_2 balance: credit the colonic bacteria," *Gastroenterology,* 102, 1992, pp. 1424–6.

Sun, Q. et al., "Hydrogen-rich saline protects myocardium against ischemia/reperfusion injury in rats," *Exp Biol Med*, 234, 2009, pp. 1212 – 1219.

Taille, C. et al., "Mitochondrial respiratory chain and NAD(P)H oxidase are targets for the antiproliferative effect of carbon monoxide in human airway smooth muscle," *J. Biol. Chem.*, 280, 2005, pp. 25350–25360.

Tannenbaum, J.S. et al., "Activation by nitric oxide of an oxidative-stress response that defends Escherichia coli against activated macrophages," *Proc. Natl. Acad. Sci.*, U. S. A., 90, 1993, pp. 9993–9997.

Tasaka, S., Amaya, F., Hashimoto, S., and Ishizaka, A., "Roles of oxidants and redox signaling in the pathogenesis of acute respiratory distress syndrome," *Antioxid. Redox Signal.*, 10, 2008, pp. 739-753.

Testard I, Ricoul M, Hoffschir F, Flury-Herard A, Dutrillaux B, Fedorenko B, et al., "Radiation-induced Chromosome Damage in Astronauts' Lymphocytes," *Int. J. Radiat. Biol.*, 70, 1996, pp. 403–11.

Tobin, B.W., Uchakin, P.N., Leeper-Woodford, S.K., "Insulin secretion and sensitivity 202 in space flight: diabetogenic effects," *Nutrition*, 18, 2002, pp. 842–8.

Turner, James E., *Atoms, Radiation, and Radiation Protection.* John Wiley & Sons, Inc., 2nd ed., 1995. pp. 421-422.

Watson, T., Goon, P.K., and Lip, G.Y., "Endothelial Progenitor Cells, Endothelial Dysfunction, Inflammation, and Oxidative Stress in Hypertension," *Antioxid. Redox Signal.*, 10, 2008, pp. 1079–1788.

Wei, Y.H., Lu, C.Y., Wei, C.Y., Ma, Y.S., and Lee, H.C., "Oxidative stress in human aging and mitochondrial disease consequences of defective mitochondrial respiration and impaired antioxidant enzyme system," *Chin. J. Physiol.*, 44, 2001, pp. 1-11.

Whiteman, M., et al., "The Novel Neuromodulator Hydrogen Sulfide: An Endogenous Peroxynitrite 'scavenger'?," *J. Neurochem.*, **90**, 2004, pp. 765-768.

Xie, K., Yu, Y., Pei, Y., Hou, L., Chen, S., Xiong, L., Wang, G., "Protective Effects of Hydrogen Gas on Murine Polymicrobial Sepsis via Reducing Oxidative Stress and HMGB1 Release," *Shock*, 2009

Zuckerbraun, B.S. et al., "Carbon monoxide signals via inhibition of cytochrome c oxidase and generation of mitochondrial reactive oxygen species," *FASEB J.*, 21, 2007, pp. 1099–1106.