USE OF ANIMAL MODELS FOR SPACE FLIGHT PHYSIOLOGY STUDIES, WITH SPECIAL FOCUS ON THE IMMUNE SYSTEM

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ABSTRACT

Animal models have been used to study the effects of space flight on physiological systems. The animal models have been used because of the limited availability of human subjects for studies to be carried out in space as well as because of the need to carry out experiments requiring samples and experimental conditions that cannot be performed using humans. Experiments have been carried out in space using a variety of species, and included developmental biology studies. These species included rats, mice, non-human primates, fish, invertebrates, amphibians and insects. The species were chosen because they best fit the experimental conditions required for the experiments. Experiments with animals have also been carried out utilizing ground-based models that simulate some of the effects of exposure to space flight conditions. Most of the animal studies have generated results that parallel the effects of space flight on human physiological systems. Systems studied have included the neurovestibular system, the musculoskeletal system, the immune system, the neurological system, the hematological system, and the cardiovascular system. Hindlimb unloading, a ground-based model of some of the effects of space flight on the immune system, has been used to study the effects of space flight conditions on physiological parameters. For the immune system, exposure to hindlimb unloading has been shown to results in alterations of the immune system similar to those observed after space flight. This has permitted the development of experiments that demonstrated compromised resistance to infection in rodents maintained in the hindlimb unloading model as well as the beginning of studies to develop countermeasures to ameliorate or prevent such occurrences. Although there are limitations to the use of animal models for the effects of space flight on physiological systems, the animal models should prove very valuable in designing countermeasures for exploration class missions of the future.

INTRODUCTION AND OVERVIEW

Animal models have been used extensively to study the effects of spaceflight on physiological conditions. The rat has been the animal used most extensively, but some studies have also been carried out utilizing mice and rhesus monkeys (Sonnenfeld, 2005). Invertebrates have also been extensively used, as have fish and amphibians. Studies have been carried out on just about every physiological parameter that could be studied, including the musculoskeletal system, the cardiovascular system, neurovestibular system, the immune system, and overall developmental biology.

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Animal models have been most useful when carrying out experiments that could not be carried out on human subjects (Sonnenfeld, 2005). These experiments involve exposure to conditions that would be dangerous for humans. Also, developmental biology studies have been carried out in animals flown in space that could not have been accomplished using humans. Additionally, since the number of crewmembers is small and their time is extremely limited, animal models have been utilized to provided preliminary data on which to base future human studies. Therefore, animal models have proven to be extremely useful in providing information on detrimental effects of space flight conditions on physiological function as well as for the development of countermeasures to ameliorate of prevent such detrimental effects. No doubt, animal models will play a crucial role in the realization of the initiative that has been announced to develop exploration class missions for the exploration of space.

The nature of the changes induced by space flight in animal physiological system has been included in a definitive review volume, and they will not be replicated here (Sonnenfeld, 2005). Instead, recent work using animal to study the effects of space flight conditions on the immune system will be highlighted in this review. Additional background information on the overall use of animals to study the effects of space flight on physiology can be found in the review volume (Sonnenfeld, 2005).

Additionally, since the opportunities to carry out space flight animal studies are rare and expensive, ground-based systems have been developed that model some of the effects of space flight on animal physiology (Giron et al., 1967; Il'in and Novikov, 1980; Morey, 1979; Musacchia et al., 1980; Space Studies Board, 1998). The need for these models has been heightened as we have embarked upon the era of planning for exploration class space missions (Sonnenfeld, 2005). The limited ability to carry out space flight animal experiments during the construction phase of the international Space Station compounded by the difficulties in carrying out flight experiments when the activities of the Space Shuttle fleet are limited or suspended, has enhanced our need for the ground-based models. Without them, it seems unlikely that sufficient information will be available to allow for planning of human exploration class space flight missions.

There are several ground based animal models available, including low pressure chambers and centrifugation hypergravity models (Giron et al., 1967; Oyama and Platt, 1965) for studying the continuum of the force of gravity, but the most commonly utilized ground based model is hindlimb unloading of rodents (Il'in and Novikov, 1980;

Morey, 1979; Musacchia et al., 1980; Space Studies Board, 1998). This model is also known as hypokinetic, hypodynamic antiorthostatic suspension or tail suspension. In the model, rats or mice are suspended so that their hindlimbs are not weight bearing and with a head down tilt so that there is a fluid shift to the head. The forelimbs remain load-bearing. This results in conditions similar to some of those observed during space flight, and effects on physiological systems are most often similar to those observed during space flight (Il'in and Novikov, 1980; Morey, 1979; Musacchia et al., 1980; Space Studies Board, 1998). Chapes et al., (1993) reviewed the effects of hindlimb unloading on the immune system and showed that hindlimb unloading was an acceptable, but not perfect, model for the effects of space flight on the immune system. Most of the effects of space flight on the immune system were replicated using the hindlimb unloading model, with the exception of leukocyte subset distribution in rats.

SPACE FLIGHT CONDITIONS, GROUND-BASED MODELS AND THE IMMUNE SYSTEM

Exposure to space flight has been shown to modify many immunological parameters (Sonnenfeld and Shearer, 2002; Sonnenfeld, Butel and Shearer, 2003). These parameters have also been recently extensively reviewed, and have been demonstrated using rats, mice and rhesus monkeys. In brief, the immunological factors shown to be affected by space flight in animals include: leukocyte subset distribution, leukocyte blastogenesis, the response of bone marrow cells to colony stimulating factors, cytokine production, natural killer cell activity, and neutrophil activity. The development of these immunological responses in offspring of pregnant rats flown in the Space Shuttle were not affected (Sonnenfeld and Shearer, 2002; Sonnenfeld, Butel and Shearer, 2003).

Exposure of rodents to the hindlimb unloading model has also resulted in changes in the immune system similar to those observed during space flight (Sonnenfeld and Shearer, 2002; Sonnenfeld, Butel and Shearer, 2003). Again, these have been recently reviewed (Sonnenfeld and Shearer, 2002; Sonnenfeld, Butel and Shearer, 2003), and details will not be given here. Functional immune responses have been shown to be altered after rodents have been placed in the hindlimb unloading model, including the same functional immune response that have been shown to be affected by space flight such as cytokine production, leukocyte blastogenesis, response of bone marrow cells to colony stimulating factors, neutrophil activity, and resistance to infection. Leukocyte subset distribution was not affected in the same way by hindlimb unloading as it was by space flight, suggesting that the model is not useful for lymphocyte cell distribution studies (Sonnenfeld and Shearer, 2002; Sonnenfeld, Butel and Shearer, 2003).

SPACE FLIGHT CONDITIONS, GROUND-BASED MODELS AND RESISTANCE TO INFECTION

Although it is clear that exposure of animals to space flight conditions results in alterations of immunological parameters, the question can still be asked, does this affect the actual health and well-being of the host? This question could only be answered by carrying out studies using infectious disease or tumor models. For obvious reasons, such studies could not be carried out using humans. Animal models have proved invaluable for carrying out infectious disease studies and have increased our understanding of the biological significance of the effects of space flight conditions on the immune system.

Since space flight experimental opportunities for animal studies have been so limited, infection studies have not been carried out in space. Therefore, the hindlimb unloading model has proven to be invaluable for carrying out experiments on the effects of space flight conditions on resistance to infection (Table 1).

Pathogen	Effect of Hindlimb Unloading	Reference
Encephalomyo-carditis virus D	Compromised resistance	Gould et al., 1987
Propionibacterium acnes	Decreased innate immunity	Fleming et al., 1990
Listeria monocytogenes	Enhanced innate resistance, compromised acquired immunity and resistance	Miller and Sonnenfeld, 1993, 1994
Klebsiella pneumoniae	Compromised resistance; Could be protected if pretreated with active hexose correlated compound (AHCC)	Belay et al., 2002; Aviles et al., 2003b, 2004
Pseudomonas aeruginosa	Compromised resistance	Aviles et al., 2004

Table 1. Effect of hindlimb unloading of mice on resistance to infection.

In an early study, female Swiss/Webster mice were inoculated with the D variant of encephalomyocarditis virus (EMC-D virus). Females of the Swiss/Webster strain normally are totally resistant to infection with EMC-D virus (Gould et al., 1987) with the resistance mediated, at least in part, by interferon (Gould et al., 1987). Hindlimb-unloaded mice became susceptible to infection, whereas mice that were restrained without head down tilt and carrying full load on their hind limbs were still resistant to infection (Gould et al., 1987). The ability of mice to produce interferon-α/β correlated with the alteration in resistance to EMC-D virus.

An additional experiment using mice subjected to hindlimb unloading was carried out by Fleming et al., (1990). The results of the study showed that hindlimb-unloaded mice had impaired ability to produce superoxide, decreased ability to kill phagocytosed bacteria (Propionibacterium acnes), and altered corticosterone levels. This suggested a compromise of innate immune defenses, particularly neutrophils, by hindlimb unloading.

Mice that were placed in the hindlimb unloading model showed enhanced resistance to primary infection with Listeria monocytogenes, an intracellular pathogen (Miller and Sonnenfeld, 1993, 1994). This was probably due to enhanced macrophage function and production of cytokines produced by macrophages that resulted in enhanced clearance of the pathogen (Miller and Sonnenfeld, 1993, 1994). At the same time that the resistance to the primary L. monocytogenes infection was strengthened by hindlimb unloading, the ability to generate long-term T cell mediated immunologic memory to the L. monocytogenes was inhibited (Miller and Sonnenfeld, 1993, 1994). Therefore, although initial resistance to an obligate intracellular organism was enhanced by hindlimb unloading, the ability to develop long-term T cell-mediated resistance to challenge with the organism was inhibited (Miller and Sonnenfeld, 1993, 1994). Therefore, it is possible that in space flight conditions, although initial resistance to some organisms might be enhanced, over the long-term, that resistance would be likely to be compromised.

Although the previous studies generated evidence that resistance to infection could be compromised by hindlimb unloading, the organisms studied were not likely to be pathogens during a space flight mission. EMC-D virus and L. monocytogenes are excellent organisms for studying resistance to infections in models, but they are not pathogens for humans that would be found in space flight conditions. Therefore, additional studies were carried out using potential pathogens.

Klebsiella pneumoniae is a gram negative enteric bacteria that is a major problem in immunocompromised surgical patients (Polk et al., 1992). It is part of the normal microbiota in the intestine, and when the host is immunocompromised, can spread from the gut to cause sepsis and death (Polk et al., 1992). Therefore, it is a possible pathogen form space travelers who might develop a compromised immune system. Pseudomonas aeruginosa is an opportunistic gram negative pathogen that has already caused infectious difficulties during a space flight mission (Hawkins and Ziegelschmid, 1975; Taylor, 1974). One of the astronauts in the Apollo 13 mission developed a urinary tract infection with P. aeruginosa during the mission (Hawkins and Ziegelschmid, 1975; Taylor, 1974). For these reasons, these two organisms were chosen for additional studies on the effects of hindlimb unloading on resistance to infection.

A study was undertaken to determine the effects of hindlimb unloading on resistance of mice to infection with K. pneumoniae (Belay et al., 2002). Mice were infected with K. pneumoniae. Mortality, as expected, was 50% for control mice that were normally caged and housed and received 1 LD_{50} (lethal dose for 50% of the animals) of K. pneumoniae. Mortality was 43% for control mice that were restrained and received 1 LD_{50} of K. pneumoniae., which was not significantly different from control mice. Mortality was 86% for experimental mice that were hindlimb-unloaded and received 1 LD_{50} of K. pneumoniae, which was a statistically significant different from the other groups of mice (Belay et al., 2002). Mean time to death was also significantly reduced in the hindlimb-unloaded mice. Additionally, the hindlimb-unloaded mice could not readily clear the bacteria from the blood and other organs, suggesting that the innate immune system was compromised in this infection (Belay et al., 2002). Therefore, it appears that exposure of mice to hindlimb unloading decreased resistance K. pneumoniae.

Additional studies were also completed to determine the effects of hindlimb unloading on resistance of mice to P. aeruginosa (Aviles et al., 2003a). Hindlimb-unloaded mice had significantly enhanced mortality when infected with P. aeruginosa in a fashion similar to that obtained with K. pneumoniae (Aviles et al., 2003a). The hindlimb-unloaded mice that were infected had a prolonged elevation of corticosterone, indicating that a stress response might play some role in decreasing resistance to infection (Aviles et al., 2003a).

If resistance to infection is compromised in mice by hindlimb unloading, could a countermeasure be developed that could minimize the deleterious effects of hindlimb unloading on resistance to infection? This is a question of some importance, because it is possible that such a countermeasure could be further developed to protect humans from infections during space flight missions.

Studies were begun with active hexose correlated compound (AHCC). AHCC is an extract prepared from co-cultured mycelia of several species of Basidiomycete mushrooms (Kidd, 2000). It contains polysaccharides, amino acids, and minerals and is orally bioavailable. It has been shown to have a beneficial effect on the immune

system of normal humans (Matsui et al., 2002) and rodents, including enhancement of natural killer cell activity (Burikhanov et al., 2000; Matsushita et al., 1998; Wakame et al., 1999; Yagita et al., 1998, 2002). AHCC is available "over-the-counter" as a nutritional supplement).

In the study carried out with AHCC, mice pre-treated for one week by gavage with AHCC and then hindlimb-unloaded with continued AHCC treatment were significantly protected from infection with K. pneumoniae (Aviles et al., 2003b). In fact, protection against infection was greater in hindlimb-unloaded mice than in normally caged mice, suggesting that more beneficial effects would be observed in immunocompromised hosts (Aviles et al., 2003b). This surprising result stimulated additional mechanistic studies with AHCC. Hindlimb-unloaded mice infected with K. pneumoniae could not clear the infection from the blood, but hindlimb-unloaded mice pre-treated with AHCC and infected with K. pneumoniae were able to clear the bacteria from the blood (Aviles et al., 2003b). These results suggested that the immune system was stimulated by pre-treatment with AHCC, resulting in clearing of the infection.

Additional mechanistic studies have been carried out to determine the role of AHCC in enhancing resistance to infection. Hindlimb–unloaded mice infected with K. pneumoniae and pre-treated with AHCC showed enhanced cytokine production, spleen cell blastogenesis and peritoneal cell nitric oxide production (Aviles et al., 2004). These results again supported that AHCC treatment enhanced innate immunity in immunocompromised hosts, but also showed a possibility that AHCC treatment could enhance acquired immunity (Aviles et al., 2004).

CONCLUSIONS

The results of the infectious disease studies show that exposure of mice to hindlimb unloading conditions resulted in compromised immune responses and compromised resistance to infection. AHCC may be a useful countermeasure to protect mice from the compromising effects of hindlimb unloading on resistance to infection. This certainly raises an issue that will require further study in actual space flight experiments.

The above results could not have been obtained without the use of the hindlimb unloading animal model. The space flight studies that should be carried out will not be able to be completed without the use of animals. The countermeasures that must be developed must have their efficacy and safety tested in animal models.

The need for these models is not limited to the immune system. Similar problems as well as the need for development of countermeasures exist in all disciplines (Sonnenfeld, 2005). Therefore, it is extremely important, as we go forward with planning for exploration class human space flight missions, that we include the use of both ground-based and flight animal models to enhance our ability and enable u to protect future space travelers from any detrimental effects of the space flight environment.

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