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Wound Healing and Mucosal Immunity During Short Mars Analog Environment Mission: Salivary Biomarkers and Its Clinical Implications

Kısa Mars Analog Çevre Misyonu Sırasında Yara İyileşmesi ve Mukozal Bağışıklık: Tükürük Biyomarkerları ve Klinik Etkileri

Balwant Rai[1], Jasdeep Kaur[2], Bernard. H. Foing[1]

[1]Earth&Life Sciences, Vrije Universiteit Amsterdam&ILEWG, Amsterdam, The Netherlands
[2]JBR Institute of Health Education Research and Technology, Punjab, India

Abstract

Objective: Wound healing in an extreme environment with microgravity is not well characterized, despite the likelihood that the increasing use of manned spaceflight as a research and commercial enterprise raises the probability of traumatic injury in this state. Hence, this study was conducted to determine the impact of the isolated environment of the Mars Desert Research Station on mucosal immunity and wound healing.

Materials and Methods: Two punch biopsy wounds were placed on the hard palate of two crewmembers. The first wound was made during summer vacation, whereas the second was placed on the contralateral side 3 days before the Mars analog mission began. Thus, each crewmember served as his/her own control. Two independent methods were used to assess healing. A ten-item perceived stress scale, salivary cortisol, Immunoglobulin A, IgG and IgM were measured.

Results: There were significant differences in the proportion of the wound size healed between vacation and the mission. Salivary IgA, IgM, IgG and cortisol levels showed significant differences between vacation and mission.

Conclusion: These data suggest that stress can have significant consequences for wound healing. The effects of stress on wound repair could have important clinical implications, including for recovery from surgery.

Key Words: Cortisol, IgA, IgM, IgG, Saliva, Wound healing

Özet

Amaç: Araştırma ve ticari amaçlı olarak insanlı uzay uçuşu kullanımının artmasıyla, bu durumda travmatik yaralanma ihtimalinin yükselmesine karşın mikrogravite olan uç seviyede bir ortamda yara iyileşmesi iyi bir şekilde karakterize edilmemiştir. Dolayısıyla, bu çalışma Mars Çöl Araştırma İstasyonunda izole bir ortamın mukozal bağışıklık ve yara iyileşmesi üzerine etkisini belirlemek amacıyla yapılmıştır.

Gereç ve Yöntem: İki mürettebatın sert damağına iki biyopsi yarası açıldı. İlk yara, yaz tatili sırasında ikincisi ise Mars analog misyonu başlamadan 3 gün önce kontra-lateral tarafına açıldı. Böylece, uçuş ekibinin her biri kendi kontrolü olarak değerlendirildi. İyileşmelerin değerlendirilmesinde İki bağımsız yöntem kullanılmıştır. On maddelik algılanan stres ölçeği, tükürük kortizol, Immunglobulin A, IgG ve IgM ölçüldü.

Bulgular: Tatil ve misyon arasında iyileşmiş yara büyüklüğü oranı açısından anlamlı fark bulunmuştur. Tükürük IgA, IgM, IgG ve kortizol seviyeleri tatil ve misyon sırasında anlamlı farklılık göstermektedir.

Sonuç: Bu veriler stresin yara iyileşmesi üzerinde önemli sonuçları olabileceğini düşündürmektedir. Yara tamiri üzerine cerrahi sonrası toparlanma dahil stresin önemli klinik etkileri olabilir.

Anahtar Kelimeler: IgA, IgM, IgG, Kortizol, Tükürük, Yara iyileşmesi

Introduction

The buoyancy of humans during extreme space environments has been demonstrated during missions to and around the moon [1]. A mission to Mars, however, requires humans to adapt to systemic and complex environments that stress the human body's capacity. Astronauts will face both physiological and psychological extremes during the journey to Mars, while on its terrain, and during the return to Earth [2]. The effect of microgravity and the space environment during short- and long-duration space missions has important medical and health implications for astronauts [1-5]. However, little is known about wound healing in an extreme environment with microgravity, despite the likelihood that the increasing

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Correspondence to: Balwant Rai, Faculty of Earth&Life Sciences, Vrije Universiteit Amsterdam&ILEWG, Amsterdam, The Netherlands
Phone: +31 20 59 89898 e-mail: drbalwantraissct@rediffmail.com

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use of manned spaceflight as a research and commercial enterprise raises the probability of traumatic injury in this state. Published studies about wound repair in space have been confined to the repair of bone or muscle, which are two tissues that depend on biomechanical stimulation for their proper repair [6-8].

Oral wounds are fairly common mucosal wounds; healing in these tissues can provide insight into the susceptibility of other mucosal tissue to infection and their subsequent ability to repair. Reinstating the physical barrier provided by intact epithelium protects the host from infection after wounding and requires the orchestration of both recruited inflammatory and immunological cells and indigenous tissue cells [9, 10]. It has been reported that stress can alter multiple aspects of immune function, including the production of pro-inflammatory cytokines that are important for wound repair. Because neutrophil function is also impaired by stress [9], distressed individuals face additional risks from infection after wounding. The stressors in Antarctica (an isolated and extreme environment) are amplified by isolation and anxiety and have been shown to have a negative effect on the Cell-Mediated Immune (CMI) response [11]. To best of our knowledge, no study has been published on the effects of the isolated environment of the Mars Desert Research Station on oral mucosal immunity and wound healing. Hence, this study was conducted to determine the impact of the isolated environment of the Mars Desert Research Station on oral mucosal wound healing.

Materials and Methods

The two crewmembers were selected from the 100B ILEWG EuroMoonMars mission as EuroMoonMars was organised by ILEWG. One female crewmember was aged 26.6 years, and one male crewmember was aged 27.7 years. One male crewmember from crew-78 of the MDRS in the USA was selected. The average caloric intake and average calcium intake of the crewmembers during the mission was 2400 kcal/day (range 2090-3200 kcal/day) and 1267 mg/day (1130-1400 mg/day), respectively. Dietary sodium and potassium intake was maintained at 98 (80-103) and 86 (75-120) mmol/day, respectively. Water intake was ad libitum, and the average water intake was 1236 (68) mL/day. Written informed consent was obtained from the subjects. The first wound was placed during summer vacation, just before the beginning of the selection procedure for the mission (MDRS). The side of the hard palate the first wound was placed on was determined by a coin toss. Approximately 20 weeks later, the second wound was placed on the contralateral side 2 days before the start of the mission.

We followed standardized procedures for wound creation and assessment for 14 days [12]. The hard palate was anesthetized using 2% Xylocaine, and a 3.5 mm wound was outlined between the palatal roots of the first and second molars, using an oral punch. A scalpel was then used to remove the surface epithelium and underlying superficial connective tissue, leaving a wound approximately 1.8 mm deep. The wound was not dressed. The subjects were instructed not to use rinses or other special home care other than normal oral hygiene. The size of the wound was assessed using a photograph obtained with an intraoral camera. A standard-sized label placed adjacent to the wound was used to account for magnification and angulation errors. The images were blindly coded, and the wound and the standardized labels were measured. As the wound approached closure, which was determined by visual inspection (after at least day 5), the area was blotted dry with a gauze sponge, and 3% hydrogen peroxide was applied to the wound. The wound was considered negative for peroxide bubbling if it was negative for 2 consecutive days. The peroxide test for wound healing measures the quality of the epithelial barrier. If the epithelial barrier is intact, then the hydrogen peroxide does not diffuse into the connective tissue, and little oxygen is liberated. If the barrier is incomplete, then bubbles of oxygen are visible on the surface of the wound. We have used this method in the skin model and in a mouse model and it correlates well with wound size measurements. The ten-item perceived stress scale, administered at the time of the biopsy and every day up to day 14, measured the degree to which subjects perceived their daily life during the preceding week as unpredictable, uncontrollable, and overloaded. Subjects rated each item from 0 (never) to 4 (very often) [13]. Saliva samples were collected every day up to day 1. The samples were immediately frozen at -4C, centrifuged and analyzed for biomarkers.

Salivary cortisol (Salimetrics Inc., PA, USA) was measured. Salivary unstimulated Immunoglobulin A, IgG and IgM were measured using ELISA kits (Biosource International, Camarillo, CA, USA). IL-1β levels were assessed using enzyme-linked immunosorbent assay (ELISA). Data were analyzed using SPSS, version 11 (SPSS, Chicago, IL, USA).

Results

Figure 1 shows differences in wound size between the summer vacation and mission time examinations, with the size of the wound expressed as a percentage of the initial (day 1) wound size for each set. The proportion of the wound area that was healed showed significant differences between vacation and mission time. During vacation, it took crewmembers an average of 15.78 days for the wound to heal completely, compared with 21.23 days during the mission.

Salivary IgA, IgM, IgG and cortisol levels showed significant differences between vacation and mission time (Figure 2-6). The average crewmember's IL-1β level declined an average of 68% during the mission. The crewmembers reported significantly more stress on the Perceived Stress Scale during the mission than during summer vacation. The mean stress level was 7.67 at the time of the first wound and 6.09 3 days later; in contrast, scores increased sharply before and during missions. There was good correlation between wound healing and IgA, IgM, IgG, cortisol, IL-1β (r=0.57, r=0.57, r=0.58, r=0.67, r=0.87, respectively). Additionally, we found good correlation between the perceived stress scale and cortisol level (r=0.76).

Discussion

The average time of wound healing was longer during the mission, which is consistent with the results of other studies [10, 12]. This impaired healing is due to stress, which is induced from an isolated, international, multi-cultural work environment [14, 15]. Salivary IgA, IgM, IgG and cortisol levels showed significant differences between vacation and mission time. This difference might be due to compromised mucosal immunity. Mucosal immunity, in connection with innate non-specific factors, is the first line of defense against antigens presented at the mucosal surface [16]. Secretory IgA (IgA), and to a lesser extent secretory IgM (IgM), antibodies play major roles in effective specific immunity [16]. Immunoglobulin G plasma cells are less common at the mucosal surface, but serum-derived and locally produced IgG antibodies are important in protection of the respiratory tract [17]. Alterations in IgG and albumin concentrations in mucosal secretions provide markers for increased membrane permeability, dehydration due to flow rate changes and sample deterioration. Salivary IgA, IgM, and IgG are well-accepted indicators of activity of the mucosal immune system and have been used to characterize the effects of many stressors. The salivary cortisol level, which is a biomarker of stress, increased during the mission [3]. Additionally, the crewmembers reported significantly more stress on the Perceived Stress Scale during the mission, which is consistent with previous studies [10, 12]. A good correlation between the perceived stress scale and cortisol level was found. A finding consistent with the group differences in wound repair was that mission members also produced less interleukin-β in response to lipopolysaccharide stimulation. There was good correlation between wound healing and interleukin-β levels, which demonstrates a cause-and-effect relationship between interleukin-1β and wound healing. Interleukin-β that is produced soon after tissue injury can regulate the production, release, and activation of metalloproteinases, which are important in the destruction and remodeling of the connective tissue matrix. Interleukin-1β also regulates fibroblast chemotaxis and the production of collagen [18] and stimulates the production of other cytokines important for wound healing [18]. The greatest differences in wound

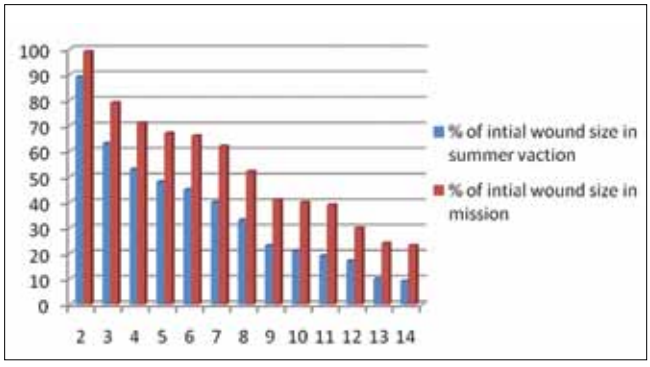

Figure 1. *Average wound size during summer vacation and mission time.*

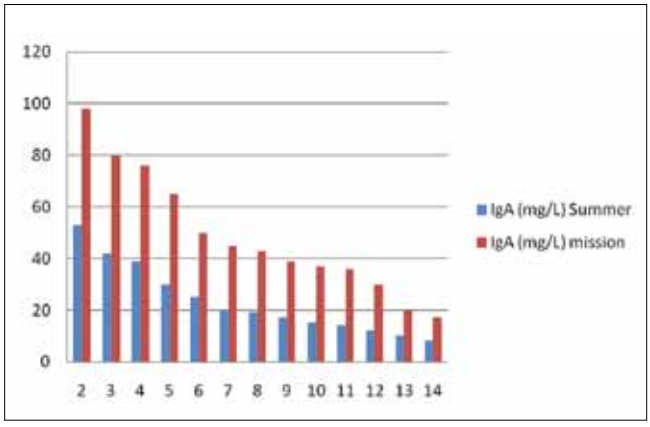

Figure 2. *Salivary IgA levels during summer vacation and during the mission.*

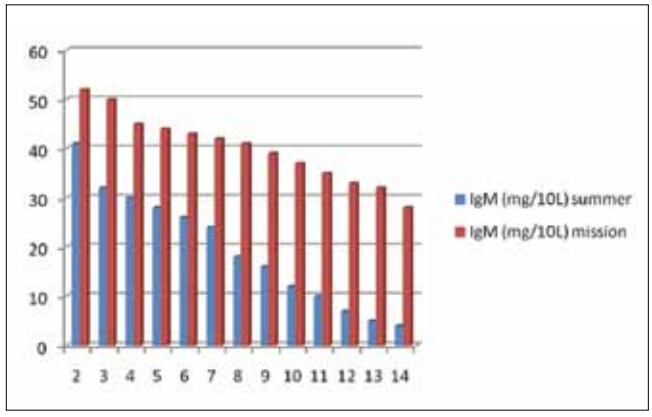

Figure 3. *Salivary IgM levels during summer vacation and during the mission.*

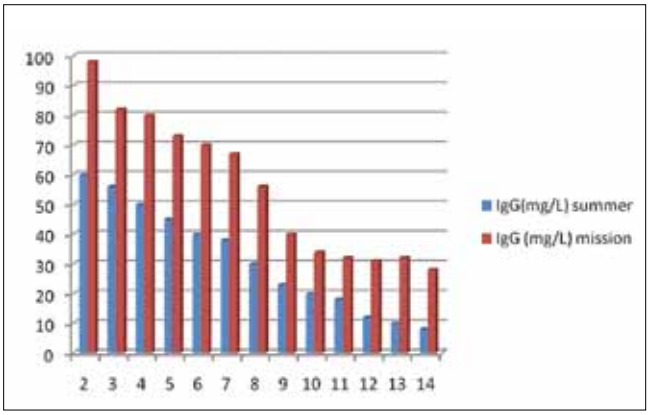

Figure 4. *Salivary IgG levels during summer vacation and during the mission.*

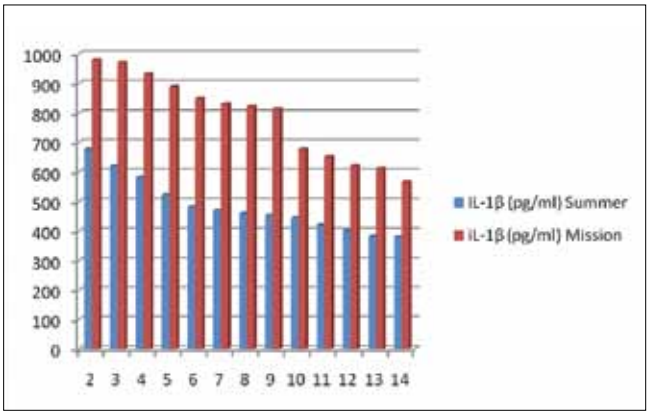

Figure 5. *Salivary IL-1β levels during summer vacation and during the mission.*

size were apparent early in the process; the differences in interleukin-1β between the two environments suggest that local production of this cytokine by leukocytes might be one immunological mechanism underlying the observed differences in wound repair. There was good correlation between wound healing and salivary IgA, IgM, IgG, cortisol, and IL-1β. Therefore, we can use these salivary markers to monitor stress and immune parameters during the selection of astronauts, during the mission and after the mission because they are noninvasive, cost-effective, less time-consuming, easy to use and non-infectious [3]. The integrity of mucosal immunity can be compromised by many factors, several of which affect expeditioners during an MDRS Mission.

This study presents the first data on mucosal immune parameters in MDRS expeditioners. The changes observed over the Antarctic season were significant, and they support the original concern that severe immune suppression occurred during time at the MDRS. The limitations of this study include its short duration, the use of only two crew-

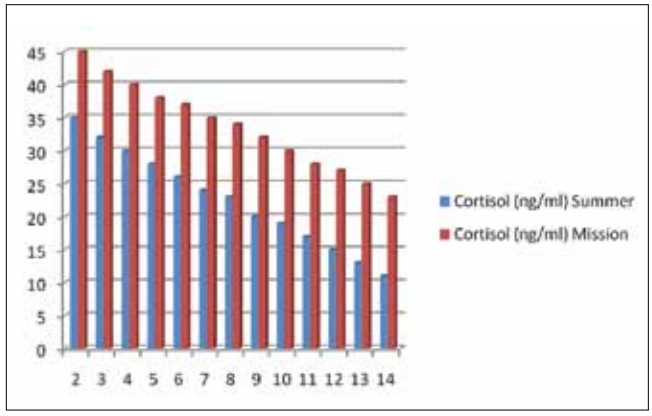

Figure 6. *Salivary cortisol levels during summer vacation and during the mission.*

members, and the fact that additional factors such as diet, sleep, and other activities were not taken into account. Thus, further studies are required to identify the causes of the changes in mucosal immunity during time at the MDRS and any implications for monitoring the health of future MDRS expeditioners.

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References

1. Antonutto G, di Prampero PE. Cardiovascular deconditioning in microgravity: some possible countermeasures. Eur J Appl Physiol 2003; 90: 283-91. [CrossRef]
2. Davis JR, Vanderploeg JM, Santy PA, Jennings RT, Stewart DF. Space motion sickness during 24 flights of the space shuttle. Aviat Space Environ Med 1988; 59: 1185-9.
3. Rai B, Kaur J. Salivary stress markers and psychological stress in simulated microgravity: 21 days in 6° head-down tilt. J Oral Sci 2011; 53: 103-7. [CrossRef]
4. Rai B, Kaur J, Catalina M, Anand SC, Jacobs R, Teughels W. Effect of simulated microgravity on salivary and serum oxidants, antioxidants, and periodontal status. J Periodontol 2011; 82: 1478-82. [CrossRef]
5. Rai B, Kaur J, Catalina M. Bone mineral density, bone mineral content, gingival crevicular fluid (matrix metalloproteinases, cathepsin K, osteocalcin), and salivary and serum osteocalcin

levels in human mandible and alveolar bone under conditions of simulated microgravity. J Oral Sci 2010; 52: 385-90. [CrossRef]
6. Ilyina-Kakueva EI, Burkovskaya TE. The microgravity effect on a repair process in M. soleus of the rats flown on Cosmos-2044. Physiologist 1991; 34: 141-2.
7. Kaplansky AS, Durnova GN, Burkovskaya TE, Vorotnikova EV. The effect of microgravity on bone fracture healing in rats flown on Cosmos-2044. Physiologist 1991; 34: 196-9.
8. Davidson JM, Aquino AM, Woodward SC, Wilfinger WW. Sustained microgravity reduces intrinsic wound healing and growthfactor responses in the rat. FASEB J 1999; 13: 325-9.
9. Martin P. Wound healing-aiming for perfect skin regeneration. Science 1997; 276: 75-81. [CrossRef]
10. Glaser R, Kiecolt-Glaser JK (eds): Handbook of Human Stress and Immunity. San Diego, CA, Academic Press, 1994.
11. Muller HK, Lugg DJ, Ursin H, Quinn D, Donovan K. Immune responses during an Antarctic Summer. Pathology 1995; 27: 186-90. [CrossRef]
12. Kiecolt-Glaser JK, Marucha PT, Malarkey WB, Mercado AM, Glaser R. Slowing of wound healing by psychological stress. Lancet 1995; 346: 1194-6. [CrossRef]
13. Cohen S, Williamson GM. Perceived stress in a probability sample of the United States. In: Spacapan S, Oskamp S, (eds). Social psychology of health. Beverly Hills, CA: Sage. 1988.
14. Rai B. Rating the mental and cognitive performance in simulated microgravity environment: MDRS. Mars Desert Research Station, Crew-78, Special Science Report 2009.
15. Rai B, Kaur J. Grieofa MO, Foing BH. JBR study of human factors in Mars Analogue: MDRS crew 100 ILEWG EuroMoonMars Crew.62nd International Astronautical Congress, Cape Town, South Africa 3-7 october 2011; IAC-11.A1.4.16.
16. Brandtzaeg P, Baekkevold ES, Farstad IN, et al. Regional specialisation in the mucosal immune system: What happens in the microcompartments? Immunol Today 1999; 20: 141-51. [CrossRef]
17. Gleeson M, Cripps AW, Clancy RL. Modifiers of the human mucosal immune system. Immunol Cell Biol 1995; 73: 397-404. [CrossRef]
18. Lowry SF. Cytokine mediators of immunity and inflammation. Arch Surg 1993; 28: 1235-41. [CrossRef]
19. Foing BH, Stoker C, Ehrenfreund P. Eds, Astrobiology field research in Moon/Mars analogue environments. I J Astrobiology 2011; 10: 137-305.