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Review Article

Space Medicine – The Next Frontier for the Heart?

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ABSTRACT

Researchers have in recent years pointed to microgravity as presenting a unique opportunity for better disease prevention and treatments. Spaceflight can induce many changes in human physiological systems. In particular, the cardiovascular system is especially affected by spaceflight due to changes at the cellular level. Endothelial cells are very sensitive to microgravity. Morphological and functional changes in endothelial cells have been extensively studied since they are believed to be the source of many cardiovascular diseases. Studies have also shown that endothelial cells play a key role in angiogenesis, which can be stimulated in a clinostat-induced microgravity environment. This is a review of studies, based on different research approaches, on human umbilical vein endothelial cells. The myriad molecular cascades and signalling pathways involving gene regulation, proteins, inflammatory response activation, alteration of endothelial behaviour, and cell senescence are highlighted. Age-related disorders experienced on earth are very similar to the changes induced in space by microgravity. As we seek solutions to medical problems, the most innovative and beneficial at present are in space medicines and therapies.

Keywords: Cardiovascular system, cell senescence, endothelial cells, microgravity, molecular cascade, space medicine

BACKGROUND

Research in Microgravity

The term "space research" has become synonymous with space exploration, especially in the field of medicine. Exomedicine refers to research and development of medical solutions in the microgravity environment of space for applications on earth, most of which was conducted on the International Space Station (ISS). Space expeditions can induce many changes in human physiological systems.

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The cardiovascular system is affected by space flight, with changes manifesting as cardiac dysrhythmias, cardiac atrophy, orthostatic intolerance, and reduced aerobic capacity (Crawford-Young, 2006). Research in this area will provide knowledge for scientists to develop novel therapies and technologies for the treatment of humans on long-duration space flight missions, as well as for the management of age-related cardiac disorders on earth.

All cells are sensitive to applied or cell-generated mechanical forces, and their transduction into biochemical signals governs many physiological processes. Endothelial dysfunction is a common feature of age-related diseases (Collins, 2011; Vasto, Candore, Balisteri, Caruso & Colonna Romano, 2007). Additionally, pathological conditions, such as cardiovascular deconditioning, are also observed in space. The most likely reasons for these disorders may be the alterations in mechanotransduction (Wu, Fannin, Rice, Wang, & Blough, 2011; Vernikos & Schneider, 2010) since endothelial cells are very sensitive to mechanical forces (Chien, 2007; Chiu & Chien, 2011; Hsiai, Blackman, & Jo, 2010; Kliche, Jeggle, Pavenstadt, & Oberleithner, 2011; Lu & Kassab, 2011; Tzima, Irani-Tehrani, Kiosses, Dejana & Schtulz, 2005; Hirase & Node, 2011; Chien, Li, & Shyy, 1998). Indeed, endothelial cells, which cover the entire inner surface of blood vessels and play a crucial role in maintaining the functional integrity of the vascular wall, are continuously exposed to various hemodynamic forces to which they respond with significant changes in gene expression and protein networks (Mammoto, Mammoto, & Ingber, 2010). Exposure to microgravity triggers all these changes, and endothelial dysfunction is the common denominator. Researchers have studied the endothelial cells lining vessels and conducted deep gene expression and protein analysis. Differentially expressed genes in a microgravity environment promote oxidative stress which, when paired with an increased production of inflammatory proteins, can lead to cardiovascular disease and accelerated cell aging. Therefore, endothelial dysfunction is an important factor for microgravity induced cardiovascular deconditioning.

In this paper, the author reviews current research on endothelial cells in cardiovascular medicine in a microgravity environment, as well as future interpretations and medical therapies.

Endothelial Cell Dysfunction

Endothelial dysfunction in a microgravity environment has been researched extensively in the past (Versari, Longinotti, Barenghi, Maier & Bradamante, 2013). Versari et al., 2013 sought to elucidate the mechanism responsible for endothelial cell dysfunction in space using a genome-wide approach (cDNA microarray) that, together with the evaluation of the cytokine release, may provide a full picture.

In this study, researchers participated in the progress 40 P mission with spaceflight of human umbilical vein endothelial cells (HUVECs), an integrated experiment (SPHINX), which consisted of 12 inflight and 12 ground-based control modules that lasted for 10 days (Figure 1).

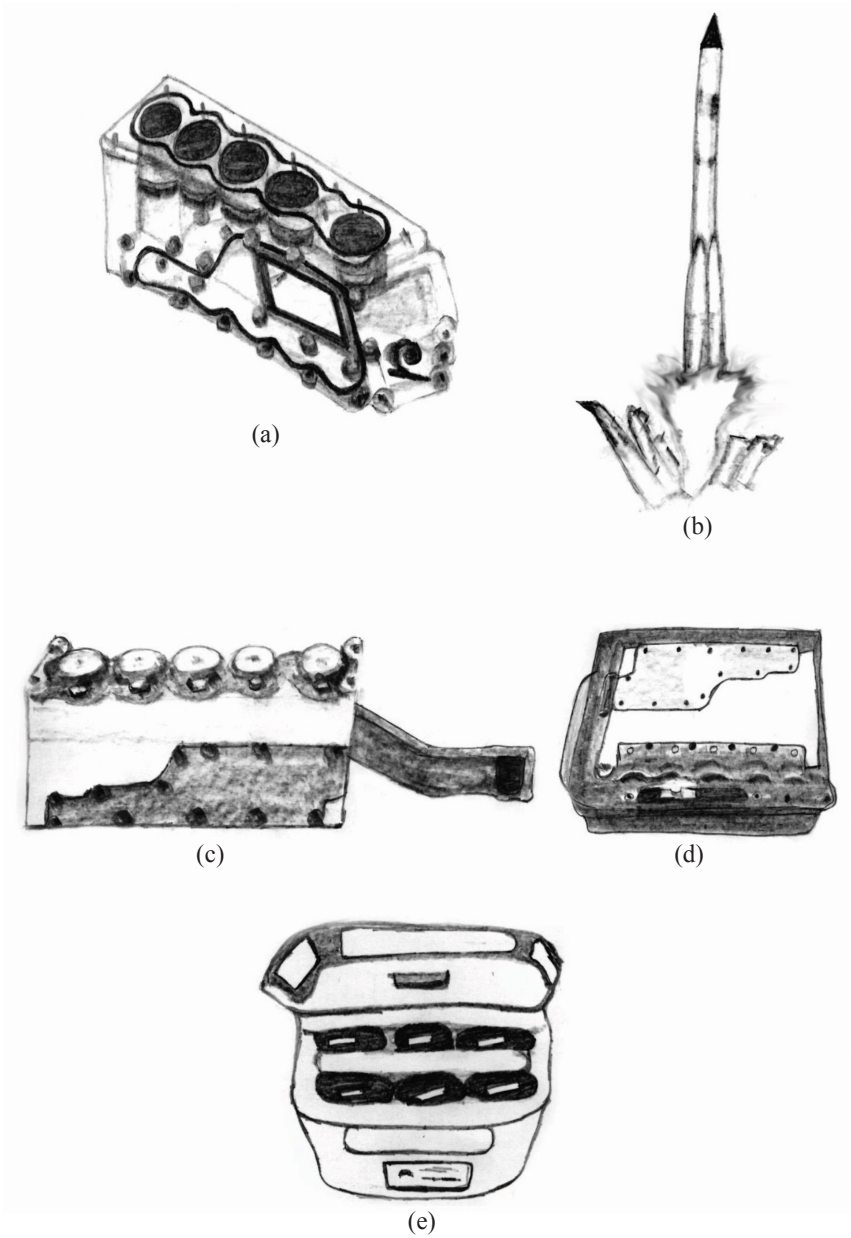

Figure 1. SPHINX experimental set-up. (a) SPHINX experiment unit. (b) Launch of the rocket carrying experiment unit. (c) SPHINX experiment unit close-up view. (d) SPHINX hardware. (e) Soft pouch to transport experimental units

Post-flight microarray analysis revealed 1023 significantly modulated genes, the majority of which were involved in cell adhesion, oxidative phosphorylation, stress responses, cell cycle, and apoptosis. A thioredoxin-interacting protein was the most upregulated, and heat shock proteins 70 and 90 were the most downregulated. Ion channels, mitochondrial oxidative

phosphorylation, and focal adhesion were widely affected. Analysis of cytokines detected in the culture media indicated a significant increase in secretion of interleukin-1α and interleukin 1β. Furthermore, nitric oxide (NO) levels were not affected. In other words, cells exposed to microgravity differentially expressed 1023 genes that altered endothelial behaviour, and promoted oxidative stress, inflammatory responses, and cell senescence.

Molecular and cellular characterisation

Kapitonova et al. (2012) conducted a study on the structures and functions of endothelial cells after a space mission using electron and laser confocal microscopy, image analysis, and MTT metabolic testing. The endothelial cells changed significantly (proliferative activity, size, contours, shape, distribution of mitochondria and microtubules) compared with control cells on Earth. These changes indicated cytoskeleton injuries and impaired barrier function of the cells, which presumably contributed to the development of endothelial dysfunction.

Additionally, morphological and functional differences were observed between endothelial cells originating from large or small vessels and between cells derived from various endothelial beds. These differences were described in terms of different responses to growth factors, expression of unique gene sets under the control of organ-specific regulatory elements, and different conditioning by the extracellular milieu (Monahan-Earley, Dvorak & Aird, 2013; Nolan et al., 2013).

Studies on the response of microvascular endothelial cells to simulated microgravity revealed important differences with macrovascular endothelial cells. No studies have been conducted using microvascular endothelial cells in real space. This led to a ground study by Balsamo et al. (2014) to define the biological and engineering requirements required to investigate cellular and molecular responses of human microvascular endothelial cells exposed to real space flight. This response was evaluated by characterising variations in gene transcription and epigenetic modifications as well as induction of genome damage and cell senescence caused by microgravity.

A recent study showed that treatment with artificial hypergravity reversed the microgravity effects induced in a model of HUVECs, thus showing potential for treating cardiovascular disease by manipulating endothelial cell phenotypes using gravitational forces (Grenon, Jeanne, Aguado-Zuniga, Conte & Hughes-Fulford, 2013). This will further assist in the identification of molecular and cellular pathways activated by space microgravity in microvascular endothelial cells, which can help in the prevention and treatment of cardiovascular disorders.

Effect on cytoskeleton

Because endothelial cells play an important role in the structure and function of the vascular wall and cardiovascular deconditioning has been observed in astronauts. Carlsson, Bertilaccio, Ballabio and Maier (2003) showed that microgravity reversibly stimulated endothelial cell growth. This effect was correlated with the overexpression of heat shock protein 70 and down-regulation of interleukin 1 alpha, a potent inhibitor of endothelial cell growth, and was also

implicated in the promotion of senescence. In addition, gravitationally unloaded endothelial cells rapidly remodelled their cytoskeleton and, after a few days, markedly down-regulated actin through a transcriptional mechanism (Carlsson et al., 2003).

Radiation effect

With advances in technological development, more manned space missions to Mars or prolonged stays on the Moon have been considered. Astronauts may be exposed to ionising radiation, cosmic rays, and solar and trapped radiation in the Van Allen belts. Space radiation can give rise to complex tissue damage that limits cellular repair and recovery (Belli, Sapora & Tabocchini, 2002; Nelson 2016).

Previous studies had examined whether mortality rates due to cardiovascular disease, cancer, accidents, and all other causes of death differed in 1) astronauts who never flew orbital missions in space, 2) astronauts who flew only in low Earth orbit (LEO), and 3) Apollo lunar astronauts, the only humans to have travelled beyond Earth's magnetosphere (Delp, Charvat, Limoli, Globus & Ghosh, 2016). The results revealed no differences in cardiovascular disease mortality rates between non-flight (9%) and LEO (11%) astronauts. However, the cardiovascular mortality rate among Apollo lunar astronauts (43%) was 4–5-fold higher than in non-flight and LEO astronauts. The long-term effect of simulated weightlessness and space-relevant total-body irradiation on vascular responsiveness in mice was also evaluated. The results showed that space-relevant irradiation induced sustained vascular endothelial cell dysfunction. Such impairment is known to lead to occlusive artery disease, and is an important risk factor for cardiovascular disease among astronauts exposed to deep space radiation.

DISCUSSION

Through SPHINX, researchers were able to study aboard the ISS the effects of microgravity on modulating the behaviour of HUVECs (Wu, Fannin, Rice, Wang & Blough, 2011; Vernikos & Schneider, 2010). This is important because microgravity rapidly promotes alterations that are similar to the ones induced by age-related diseases (Biolo, Heer, Narici, & Strollo, 2003; Nichols, Zhang, & Wen, 2006; Wang, 1999). Data showed that HUVECs were affected by spaceflight and responded by utilising mechanosensors responsible for sensing alterations in flow patterns, even though there was no flow in their environment.

Mechanotransduction is a rapidly expanding area of research because conversion of mechanical forces into biochemical signals in cells governs many physiological processes in the human body (Hoffman, Grashof, & Schwartz, 2011). It is widely recognised that defects in mechanotransduction signalling can contribute to human diseases such as immune system disorders, muscular dystrophies, and cardiomyopathies. In the specific case of endothelial cells, several mechanosensors have been proposed, from cell-to-cell adhesion and glycocalyx molecules, to ion channels, integrins, G protein-coupled receptors, and the cytoskeleton (Hsiai, Blackman & Jo, 2010). In space research, the mechanotransduction of flow in various cell types as well as in yeast is mainly observed in the cytoskeleton, which is considered as the main mechanosensor (Hughes-Fulford, 2003; Walther, Bechler, Muller, Hunzinger, & Cogoli, 1996).

Among the 1023 genes studied on the mission, the most upregulated was *TXNIP*. It is an oxidoreductase expressed in endothelial cells that controls the cell's redox status and regulates cell growth, migration, angiogenesis, and apoptosis. The TXNIP plays a key role in age-related and cardiovascular disorders since it is a critical sensor of biomechanical stress (Salminen, Ojala, Kaarniranta, & Kauppinen, 2012; Wang, Nigro, Fujiwara, Yan, & Berk, 2012; Yamawaki, Pan, Lee, & Berk, 2005; Yoshioka, Schulze, Cupesi, Sylvan, & MacGillivray, 2004; Zschauer, Kunze, Jakob, Haendeler, &Altschmied, 2011). Therefore, an overexpression of *TXNIP* can contribute to the modulation of endothelial function in space.

Oxidative stress causes extensive DNA damage and the onset of senescence in endothelial cells (Tretter, Sipos & Adam-Vizi, 2004; Martinon, 2010). The DNA damage activates the p53 signalling pathway that can lead to cell survival if the damage can be repaired or apoptosis if the damage is too severe (Liu, Chen & St. Clair, 2008), which is exactly what was observed in this study. However, the controversial results on the modulation of NO synthases and the insignificant differences found in spaceflight versus NO releases in 1-g are due to adaptation of cells after 10 days in space.

Furthermore, in studies via SPHINX, researchers were able to investigate the mechanisms responsible for alterations in HUVECs in space. They demonstrated that microgravity affected HUVECs, which subsequently responded by using the molecular machinery responsible for sensing alterations in flow patterns, even though no flow was present. It also further supported the role of oxidative stress in accelerating aging and disease.

Similarly, Shi et al., (2012) investigated the effects of microgravity on the ability of endothelial cells to foster angiogenesis. Angiogenesis is an important process by which new blood vessels form from pre-existing endothelial structures (Folkman, 1995). Previous researches have shown that proliferation, migration, and morphological differentiation of endothelial cells play critical roles in angiogenesis. NO, which is synthesized locally in the endothelium, is an important mediator of blood flow control, vascular permeability, and angiogenesis (Namkoong et al., 2008; Sessa, 2009). This study employed the use of a clinostat to simulate microgravity to permit observations of tube formation, migration, and expression of endothelial nitric oxide synthase (eNOS) in HUVECs in this environment. Significant changes were observed within 24 hours of microgravity exposure including the promotion of angiogenesis among HUVECs mediated through the P13K-Akt-Enos signal pathway.

In Malaysia, studies using HUVECs culture optimisation were conducted on microcarriers within fluid processing apparatuses that were flown to the ISS (Muid, Ali, & Nawawi, 2013). Follow-up studies were conducted on live cells after spaceflight. Results revealed that IL-6 and ICAM-1 were still elevated 3 months after space flight. However, there was a downregulation of eNOS expression in revived HUVECs. These results suggest a reduced protection of cells and the surrounding vessels, and thus may lead to hastening of atherosclerosis.

There are differences between the mechanism of cardiac deconditioning and age-related changes in microgravity. The SPHINX study clearly showed that microgravity weakened and jeopardised HUVEC function. As such, new avenues for the treatment of endothelial dysfunction in microgravity are now being researched. In addition, Shi et al. (2012) showed that simulated microgravity can have a strong effect on angiogenesis through a specific signalling pathway. Research is currently in progress to investigate the precise molecular mechanisms

responsible for the effects of simulated microgravity on the P13K signalling pathway. The Malaysian study suggested that the effects of spaceflight are not necessarily immediate after astronauts have returned to earth but may be delayed. This led to new research to explore preventive measures pertaining to atherosclerosis and its complications.

Studies in microgravity revealed the importance of molecular networks controlling signalling pathways and the numerous cascades required for cell survival. This is important in regards to planning for management of heart conditions in future space missions as well as space spin-off technologies on earth such as the tele-echocardiography machine, which was modified by the European Space Agency to study astronauts' hearts in space (Knight, 2016). The technology is now being used successfully in hospitals across the world. Looking to the future, novel innovations can be made using information from these molecular roadmaps. Thus, the study of the cardiovascular system in microgravity contributes enormously to knowledge about our future cardiac health.

CONCLUSION

In summary, human life have revolved around gravity on earth, and without it, life would be a very different experience. As such, it is inevitable that we devise methods to improvise and sustain space travellers during long journeys in space while also developing new medical technologies to benefit mankind as a whole.

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