Original article:

DIFFERENTIAL VASCULAR CELL ADHESION MOLECULE-1 EXPRESSION AND SUPEROXIDE PRODUCTION IN SIMULATED MICROGRAVITY RAT VASCULATURE

Ran Zhang[1*], Hai-hong Ran[2], Yu-ling Gao[1], Jin Ma[3], Ya Huang[1], Yun-gang Bai[3], Le-jian Lin[3]

[1] Institute of Geriatric Cardiology, Institute of Geriatric Cardiology, People's Liberation Army General Hospital, Beijing, 100853, China
[2] Department of Geriatric Hematology, Chinese People's Liberation Army General Hospital, Beijing, 100853, China
[3] Department of Aerospace Physiology, Fourth Military Medical University, Xi'an, 710032, China

* Corresponding author, e-mail: cardiomed_zhangran@yahoo.com.cn

ABSTRACT

Exposure to microgravity leads to orthostatic intolerance in astronauts and differential vascular structural and functional adaptations have been implicated in its occurrence. The present study tended to clarify the characteristics of vascular inflammation and oxidative stress in hindlimb unweighting (HU) rat vasculature. Male Sprague-Dawley rats were randomly divided into control (CON) and hindlimb unweighting (HU) groups. Three weeks later, immunohistochemistry was used to localize the expression of vascular cell adhesion molecule-1 (VCAM-1) and laser scanning confocal microscope were used to detect superoxide production. Immunohistochemical results revealed positive staining of VCAM-1 on endothelial cells in HU rat basilar and carotid arteries compared with CON, but not in abdominal aorta and femoral arteries. Meanwhile, HU increased O_2^- levels in all the layers of basilar and carotid arteries from HU rat but not in abdominal aorta and femoral arteries from HU rat. These data suggested that differential expression of VCAM-1 and O_2^- production were concomitant with the vascular adaptations to simulated microgravity and whether they participate in vascular structure and function remodeling merits further investigation.

Keywords: weightlessness, vascular cell adhesion molecule-1, superoxide, vascular remodeling

INTRODUCTION

When humans are exposed to microgravity during spaceflight, fluids shift toward the head and normal pressure gradient is eliminated. These marked hemodynamic changes occur rapidly on exposure to microgravity and could induce subsequent cardiovascular adaptations, which will lead to orthostatic intolerance in astronauts when they come back to the earth with 1 G gravity. Hindlimb unweighting (HU) rat is a widely used and reliable model to simulate the physiological effects of microgravity on humans. As reviewed by Zhang (2001), simulated microgravity induces upward and downward regulations in the structure and function of the cerebral and hindquarter arteries in HU rat. The cerebral arteries showed vascular hypertrophy, enhanced vasoconstriction (Zhang et al., 2000; Zhang, 2001) and impaired endothelium-dependent relaxation (Prisby et al., 2006); however, the abdominal aorta and femoral

arteries showed vascular hypotrophy, attenuated vasoconstriction and endothelium-dependent relaxation (Ma et al., 2000; Zhang, 2001). The endothelium-dependent nitric oxide (NO) (Siamwala et al., 2010; White et al., 2010) and ion channel mechanisms (Xie et al., 2005, 2010) have been indicated in vascular adaptation to simulated microgravity. Although much progress has been made in the past decade, the underlying mechanism of vascular adaptations to microgravity remains to be established.

Vascular wall inflammation has been regarded as an initial event of cardiovascular disorders. Vascular cell adhesion molecule-1 (VCAM-1), a cell surface protein expressed by endothelial cells (ECs), mediates lymphocyte recruitment and adherence via interaction with very late antigen-4 (Abdala-Valencia and Cook-Mills, 2006; Cook-Mills, 2002). The lymphocytes release cytokines such as IL-1β, TNF-α, IL-6 which are believed to result in vascular structural remodeling (Mahmud and Feely, 2005), higher vascular reactivity (Jimenez-Altayo et al., 2006). Then whether vascular inflammation contributes to differential vascular adaptations to microgravity? Therefore, the first purpose of the present study was to explore the characteristics of VCAM-1 expression in HU rat vasculature.

The term "oxidative stress" describes the oxidative injury caused by reactive oxygen species (ROS), including superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (•OH), NO and peroxynitrite ($ONOO^-$). ROS can oxidize lipoproteins, limit the vascular availability of NO, and promote vascular expression of cytokines and adhesion molecules (Koh et al., 2009). These radicals play the pathophysiological roles both in the maintenance of steady vessel wall conditions and in the vascular reactivity to altered blood flow or pressure settings (Lehoux, 2006). O_2^- has been indicated in regulating vascular inflammation, reactivity and nitric oxide synthase (NOS) expression in hypertensive rats (Vaziri et al., 2000). An increasing body of evidence suggests that oxidative stress is involved in the pathogenesis of many cardiovascular diseases, including hypercholesterolemia, atherosclerosis, hypertension, heart failure and diabetes. Then whether vascular oxidative stress contributes to differential vascular adaptations to microgravity? Therefore, the second purpose of the present study was to explore the characteristics of O_2^- production in HU rat vasculature.

MATERIALS AND METHODS

Animal treatment

The protocols and procedures used in this study were in accordance with Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences. 13-week-old male Sprague-Dawley rats were randomly assigned to 3-week hindlimb unweighting rat (HU) and control (CON). The head-down hindlimb unweighting model described in detail previously (Ma et al., 2003) was used to simulate microgravity in rats. The rats in HU group were maintained in about 30° head-down tilt position and housed individually under a 12:12 h light-dark circle with food and water available ad libitum at 24 ± 1 °C.

Tissue preparation

After 3 weeks of treatment, rats were anesthetized with pentobarbital sodium (40 mg/kg, ip) and dissected. Basilar arteries, common carotid arteries, abdominal aorta and femoral arteries were rapidly removed and placed in Krebs solution consisting of (in mmol/L): NaCl, 118.3; KCl, 14.7; KH_2PO_4, 1.2; $MgSO_4 \cdot 7H_2O$, 1.2; $CaCl_2 \cdot 2H_2O$, 2.5; $NaHCO_3$, 25; dextrose, 11.1; and EDTA, 0.026; pH 7.40. Parallel weight of soleus muscle-to-body mass ratio was used to test the efficacy of simulated microgravity. The arteries were divided into two parts. The artery segments for immunohistochemistry were fixed in 4 % paraform, and in addition, the artery slice from another part were prepared for detecting vascular O_2^- levels.

Immunohistochemical localization

Arterial segments from HU and CON rats to be examined for VCAM-1 expression were immediately frozen in Tissue-Tek O.C.T. embedding medium. 8 μm thick tissue sections were mounted on polylysine-coated slides. After preincubated with 20 % normal bovine serum for 30 min at room temperature to block the nonspecific antigens, the sections were incubated with rabbit polyclonal antibody against rat VCAM-1 (1:50, Santa Cruz Biotechnology) at 4 °C overnight in a moist chamber. The sections were incubated with a biotinylated secondary antibody (1:700, Santa Cruz Biotechnology) in PBS for 2 h at 37 °C after washing with PBS. After another three washes in PBS, the horseradish peroxidase was developed with 3,3-diaminobenzadine (DAB) (Roche Diagnostics, Mannheim, Germany) as the chromogen substrate. The sections were rinsed, dehydrated in ethanol, cleared in xylene, and mounted.

Detection of vascular O_2^--levels

Dihydroethidium (DHE) is the most popular probe used to detect O_2^- levels in vascular tissues and has been described in detail before (Münzel et al., 2002; Zanetti et al., 2005). Arteries for investigation were harvested carefully from the rats and immediately frozen in Tissue-Tek O.C.T. embedding medium. 30 μm thick frozen sections were prepared, and then were stained with 10 μmol/L DHE (Molecular Probes). Laser scanning confocal microscopic images were obtained after incubation in a light-protected humidified chamber at 37 °C for 30 minutes.

Drugs and reagents

All drugs and chemicals were obtained from Sigma Chemical (St. Louis, MO). Rabbit polyclonal anti-rat VCAM-1 was purchased from Santa Cruz Biotechnology (Santa Cruz Biotechnology Inc., Santa Cruz, USA). Horseradish peroxidase (HRP)-conjugated anti-rabbit IgG was the product of Invitrogen and dihydroethidium was from Molecular Probes.

RESULTS

General data

The body weight of the rats for investigation increased steadily during the three weeks. The significant lower soleus muscle mass (HU 82.63 ± 4.51 mg; CON 165.01 ± 6.58 mg, $P < 0.001$) and soleus muscle-to-body mass ratio (CON 0.40 ± 0.01 mg/g ; HU 0.21 ± 0.01 mg/g, $P < 0.001$) confirmed the efficacy of simulated microgravity and the reliability of animal model used in this study.

VCAM-1 expression in rat arteries

The representative expression of VCAM-1 in arteries is shown in Figure 1. VCAM-1 protein was undetectable in artery segments of basilar artery, common carotid artery, abdominal aorta and femoral artery from CON group (Figure 1A, C, E, G). However, strong staining localized to ECs surface in basilar artery (Figure 1B) and common carotid artery (Figure 1 D) revealed a significant increase expression of VCAM-1; but it was not the case in abdominal aorta and femoral arteries from HU rats (Figure 1 F, H).
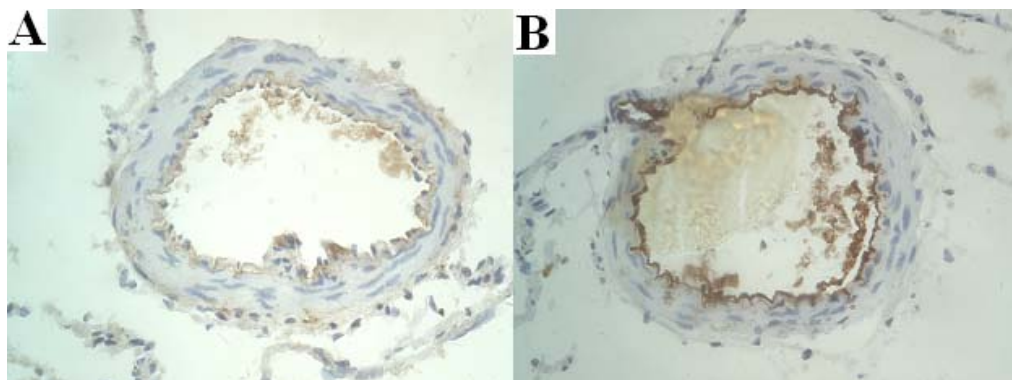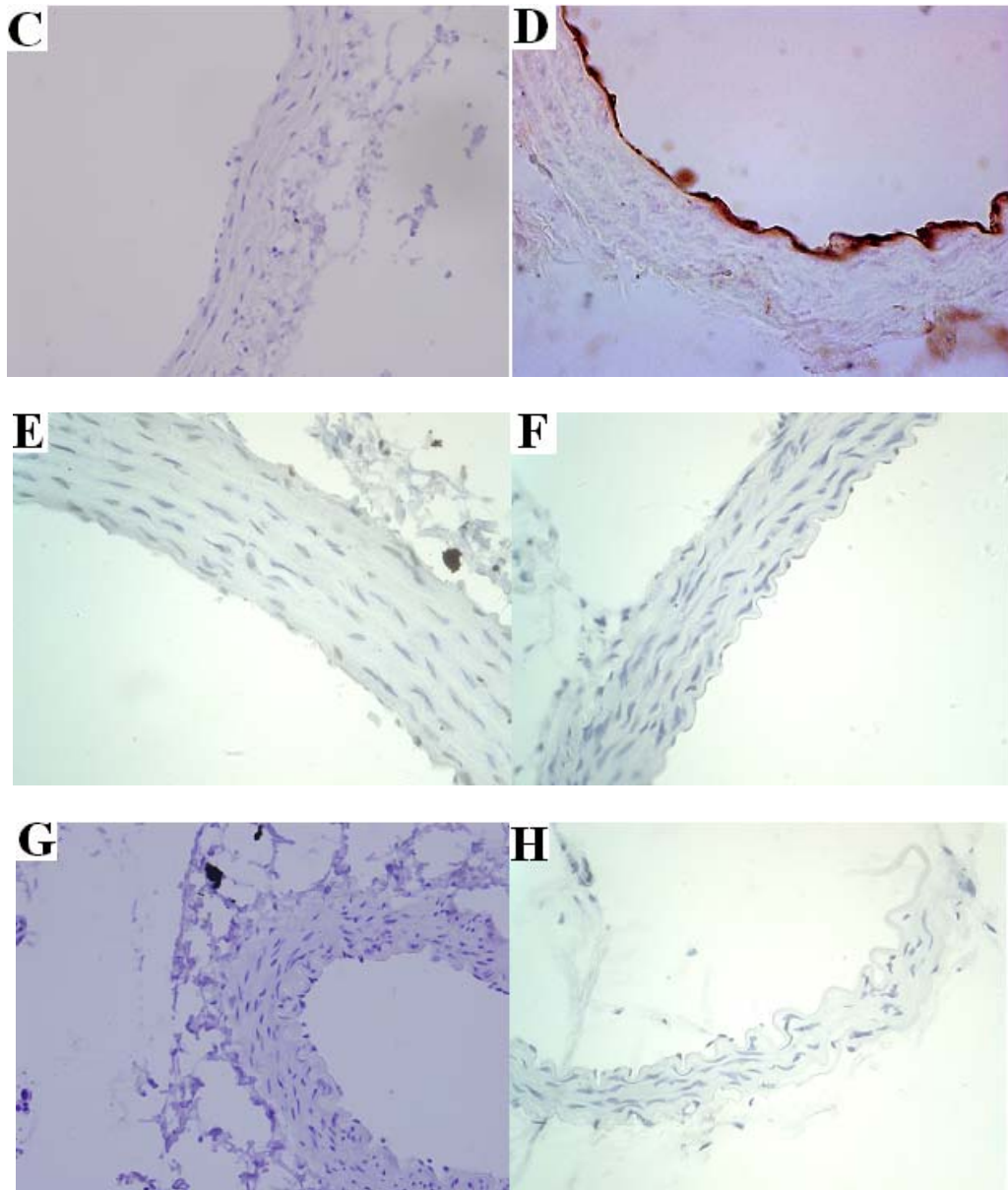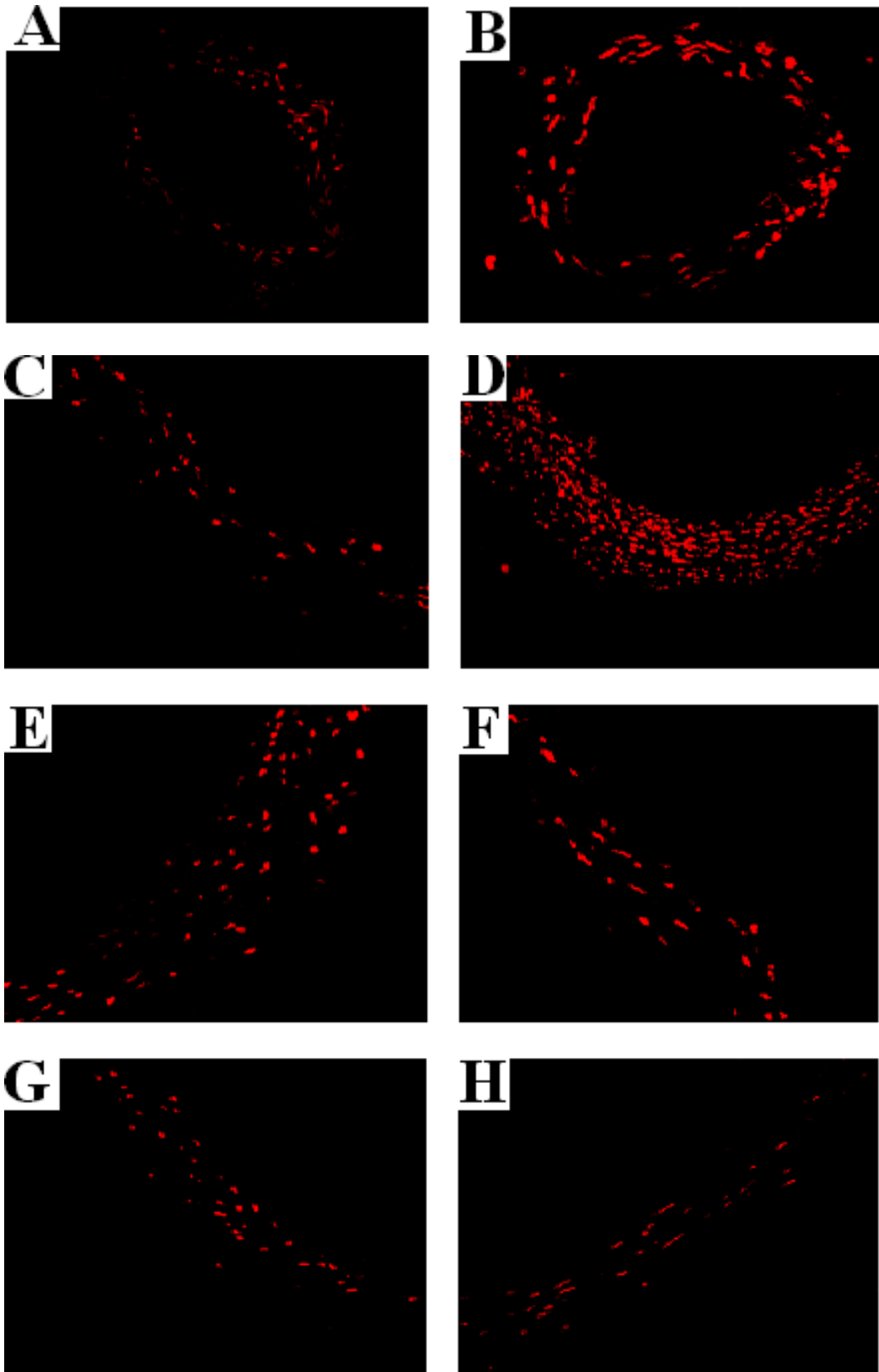
Figure 1: Representative photomicrographs of immunohistochemical examination for VCAM-1 expression in basilar artery (A, B), common carotid (C, D), abdominal aorta (E, F) and femoral arteries (G, H) from CON (A, C, E, G) and HU (B, D, F, H) rat (×400).
No staining is detectable in rat basilar artery (A), common carotid (C), abdominal aorta (E) and femoral artery (G) from CON and abdominal aorta (F) and femoral artery (H) from HU. VCAM-1 protein expression in rat basilar and common carotid artery from HU was localized predominantly to the endothelial cells.

Superoxide production in rat arteries

Superoxide levels were shown in Figure 2, weak $O_2^{\cdot-}$ signals were detected by laser scanning confocal microscope in basilar (Figure 2A), carotid arteries (Figure 2C), abdominal aorta (Figure 2E) and femoral arteries (Figure 2G) from CON rats. Compared with CON, intense $O_2^{\cdot-}$ levels were found in all layers of the basilar (Figure 2B) and carotid arteries (Figure 2D) from HU rats. However, less generation of $O_2^{\cdot-}$ was found in the abdominal aorta (Figure 2F) and femoral arteries (Figure 2H) from HU rats compared with CON.

Figure 2: Representative fluorescence photographs of rat basilar artery (A, B), common carotid artery (C, D), abdominal aorta (E, F) and femoral arteries (G, H). A, C, E, G are from CON rat and B, D, F, H are from HU rat. Arteries were labeled with the oxidative dye DHE, which would react with $O_2^{\cdot-}$ to form ethidium and produced a red fluorescence. Original magnification: ×400.

DISCUSSION

The major findings of the present study were that 3-week simulated microgravity induced by hindlimb unweighting resulted in increased VCAM-1 expression and superoxide production in cerebral and carotid arteries but not in abdominal aorta and femoral arteries.

Chronic inflammation in the vasculature is a pathogenic feature of atherosclerosis and cardiovascular disease. Although laboratory evidence found the increased expression of E-selectin or intercellular cell adhesion molecule-1 (ICAM-1) but not VCAM-1 in human umbilical vein ECs when exposed to simulated microgravity (Romanov et al., 2001), evidence from long-term gravity vector demonstrated the expression of ICAM-1, E-selectin and VCAM-1 on cultured ECs (Buravkova et al., 2005). VCAM-1 is also referred to inducible cell adhesion molecule CD106 with a molecule weight of 100–110 kDa. Hemodynamic stress (Chappell et al., 1998; Sung et al., 2007) and angiotensin II (Savoia and Schiffrin, 2006) modulates the expression of VCAM-1. As indicated by Wilkerson and associates (Wilkerson et al., 1999) the mean arterial pressure of the aorta increased nearly 21 mm Hg after 2-week simulated microgravity. Meanwhile, the content of angiotensin II (Ang II) and its receptors, the key components of rennin-angiotensin system (RAS), increased in 4-week HU rat cerebral and carotid arteries and decreased in femoral arteries (Bao et al., 2007; Meng et al., 2002). Based on the above mentioned, we hypothesized that the expression of VCAM-1 might increase in HU rat cerebral and carotid arteries. As expected, 3-week HU upregulated the expression of VCAM-1 in rat basilar and common carotid artery ECs, but not in abdominal aorta and femoral artery ECs.

Ang II, the main effector of the RAS, is one of the major mediators of vascular remodeling in cardiovascular diseases. Ang II mediates many of its physiological and pathophysiological actions by stimulating formation of intracellular ROS, which plays important roles in modulating inflammatory reactions (Cheng et al., 2005; Benigni et al., 2010; Kaneto et al., 2010). ROS are implicated at virtually every stage in the inflammatory response, including vascular permeability, leucocyte adhesion and transmigration, chemotaxis, cell growth and fibrosis (Harrison et al., 2003). NAD(P)H oxidase, mitochondrial electron transport chain, uncoupled NOS, cytochrome P450 monooxygenase and xanthine oxidase have been demonstrated the important sources of ROS in the vasculature and NAD(P)H oxidase has been indicated a major one of the wide sources (Clempus and Griendling, 2006). Ang II induces activation of vascular NAD(P)H oxidase and increases expression of NAD(P)H oxidase subunits (Cheng et al., 2005). In the present study, 3-week hindlimb unweighting increased the production of O_2^- in rat basilar and carotid arteries of all the layers, which might be associated with the activation of RAS in HU rat vasculature. Intracellular oxidative stress in vascular cells activates NF kappaB to stimulate transcription of proinflammatory genes, which results in synthesis of protein products such as cell adhesion molecules, cytokines, and chemokines (Pueyo et al., 2000). In transgenic rats, increased Ang II type I (AT1) receptor/NADPH oxidase activation/ROS contributes to vascular endothelial dysfunction and inflammation (Wei et al., 2007). Although we cannot conclude the causation between vascular inflammation and oxidative stress in the settings of simulated microgravity till now, the positive VCAM-1 expression and increased O_2^- production in 3-week HU rat cerebral and carotid arteries that we found in the present study merits further investigation.

NO modulates vascular tone, inhibits platelet function, prevents adhesion of leukocytes, and reduces proliferation of the intima (Förstermann, 2010). Endothelial dysfunction characterized by an enhanced inactivation and/or reduced synthesis of NO occurs throughout the cardiovascular disease process. This may be partly attributed by decreased bioactive NO secondary to increased O_2^- production by inactivating

NO to form ONOO⁻, which in turn, uncouple endothelial NOS to become a dysfunctional O_2^- generating enzyme that contributes to vascular oxidative stress (Marchesi et al., 2009). Inflammation and oxidative stress contribute to endothelial dysfunction while endothelial dysfunction promotes oxidative stress and inflammation (Koh et al., 2009). Localized inflammatory reaction, as well as enhanced local oxidative stress, contributes to abnormal endothelium-dependent relaxation at both coronary conduit and resistance arteries (Pendyala et al., 2009). The activation of the NF kappaB inflammatory pathway by angiotensin II-induced ROS generation contributed to systemic inflammation and endothelium dysfunction in salt-sensitive hypertension (Zhou et al., 2010). TNF-α and IL-1β inhibits the NO-dependent component of endothelium-dependent relaxation by increasing levels of O_2^- and the ability of O_2^- to inactivate NO (Wimalasundera et al., 2003). However, IL-10 limits Ang II-mediated oxidative stress and vascular dysfunction both in vitro and in vivo (Didion et al., 2009) and normalizes blood pressure and endothelial function in pregnancy-induced hypertensive rats (Tinsley et al., 2010). The differential adaptations to simulated microgravity in rat vasculature has been reviewed by Zhang (2001), and enhanced vasoconstriction and impaired endothelium-dependent relaxation in HU rat cerebral and carotid arteries have been demonstrated in previous studies (Geary et al., 1998; Wilkerson et al., 2005). Although NO-dependent mechanism (Siamwala et al., 2010; White et al., 2010) has been indicated, the underlying mechanism of endothelium-dependent relaxation in the settings of simulated microgravity remains unclear. Since NO plays a pivotal role in angiogenesis (Siamwala et al., 2010) and early changes (3-day HU) in vasoreactivity (White et al., 2010), and differential VCAM-1 expression and O_2^- production existed in HU rat arteries, whether they participate in vascular adaptations to simulated microgravity by influencing NO merits further investigation. In addition, whether vascular inflammation and oxidative stress participate in ion channel remodeling should also be clarified.

In conclusion, we observed the differential VCAM-1 expression and O_2^- production in HU rat vasculature and further evidence should be provided to clarify whether they participate in vascular remodeling. In addition, whether anti-inflammation and antioxidation treatment could reverse abnormal vascular response to agonists should be clarified.

REFERENCES

Abdala-Valencia H, Cook-Mills JM. VCAM-1 signals activate endothelial cell protein kinase Calpha via oxidation. J Immunol 2006;177:6379-87.

Bao JX, Zhang LF, Ma J. Angiotensin and AT1R expression in cerebral and femoral arteries during hindlimb unloading in rats. Aviat Space Environ Med 2007;78:852-8.

Benigni A, Cassis P, Remuzzi G. Angiotensin II revisited: new roles in inflammation, immunology and aging. EMBO Mol Med 2010;2:247-57.

Buravkova L, Romanov Y, Rykova M, Grigorieva O, Merzlikina N. Cell-to-cell interactions in changed gravity: ground-based and flight experiments. Acta Astronaut 2005;57:67-74.

Chappell DC, Varner SE, Nerem RM, Medford RM, Alexander RW. Oscillatory shear stress stimulates adhesion molecule expression in cultured human endothelium. Circ Res 1998;82:532-9.

Cheng ZJ, Vapaatalo H, Mervaala E. Angiotensin II and vascular inflammation. Med Sci Monit 2005;11:RA194-205.

Clempus RE, Griendling KK. Reactive oxygen species signaling in vascular smooth muscle cells. Cardiovasc Res 2006;71:216-25.

Cook-Mills JM. VCAM-1 signals during lymphocyte migration: role of reactive oxygen species. Mol Immunol 2002;39:499-508.

Didion SP, Kinzenbaw DA, Schrader LI, Chu Y, Faraci FM. Endogenous interleukin-10 inhibits angiotensin II-induced vascular dysfunction. Hypertension 2009;54:619-24.

Förstermann U. Nitric oxide and oxidative stress in vascular disease. Pfluegers Arch 2010;459:923-39.

Geary GG, Krause DN, Purdy RE, Duckles SP. Simulated microgravity increases myogenic tone in rat cerebral arteries. J Appl Physiol 1998;85:1615-21.

Harrison D, Griendling KK, Landmesser U, Hornig B, Drexler H. Role of oxidative stress in atherosclerosis. Am J Cardiol 2003;91:7A-11A.

Jimenez-Altayo F, Briones AM, Giraldo J, Planas AM, Salaices M, Vila E. Increased superoxide anion production by interleukin-1beta impairs nitric oxide-mediated relaxation in resistance arteries. J Pharmacol Exp Ther 2006;316:42-52.

Kaneto H, Katakami N, Matsuhisa M, Matsuoka TA. Role of reactive oxygen species in the progression of type 2 diabetes and atherosclerosis. Mediators Inflamm 2010; article ID 453892.

Koh KK, Oh PC, Quon MJ. Does reversal of oxidative stress and inflammation provide vascular protection? Cardiovasc Res 2009;81:649-59.

Lehoux S. Redox signaling in vascular responses to shear and stretch. Cardiovasc Res 2006;71:269-79.

Ma J, Zhang LN, Zhang LF. Vasoconstrictor responsiveness of hind body vascular beds is diminished in tail-suspended rats. J Gravit Physiol 2000;7:P153-4.

Ma J, Kahwaji CI, Ni Z, Vaziri ND, Purdy RE. Effects of simulated microgravity on arterial nitric oxide synthase and nitrate and nitrite content. J Appl Physiol 2003;94:83-92.

Mahmud A, Feely J. Arterial stiffness is related to systemic inflammation in essential hypertension. Hypertension 2005;46:1118-22.

Marchesi C, Ebrahimian T, Angulo O, Paradis P, Schiffrin EL. Endothelial nitric oxide synthase uncoupling and perivascular adipose oxidative stress and inflammation contribute to vascular dysfunction in a rodent model of metabolic syndrome. Hypertension 2009;54:1384-92.

Meng QJ, Zhang LF, Zhang LN, Ma J. Changes of angiotensinogen expression in arteries of tail-suspended rats. J Gravit Physiol 2002;9:87-8.

Münzel T, Afanas'ev IB, Kleschyov AL, Harrison DG. Detection of superoxide in vascular tissue. Arterioscler Thromb Vasc Biol 2002;22:1761-8.

Pendyala LK, Li J, Shinke T, Geva S, Yin X, Chen JP, King SB 3rd, Robinson KA, Chronos NA, Hou D. Endothelium-dependent vasomotor dysfunction in pig coronary arteries with Paclitaxel-eluting stents is associated with inflammation and oxidative stress. JACC Cardiovasc Interv 2009;2:253-62.

Prisby RD, Wilkerson MK, Sokoya EM, Bryan RM Jr, Wilson E, Delp MD. Endothelium- dependent vasodilation of cerebral arteries is altered with simulated microgravity through nitric oxide synthase and EDHF mechanisms. J Appl Physiol 2006;101:348-53.

Pueyo ME, Gonzalez W, Nicoletti A, Savoie F, Arnal JF, Michel JB. Angiotensin II stimulates endothelial vascular cell adhesion molecule-1 via nuclear factor-kappaB activation induced by intracellular oxidative stress. Arterioscler Thromb Vasc Biol 2000;20:645-51.

Romanov YA, Buravkova LB, Rikova MP, Antropova EN, Savchenko NN, Kabaeva NV. Expression of cell adhesion molecules and lymphocyte-endothelium interaction under simulated hypogravity in vitro. J Gravit Physiol 2001;8:P5-8.

Savoia C, Schiffrin EL. Inflammation in hypertension. Curr Opin Nephrol Hypertens 2006;15:152-8.

Siamwala JH, Majumder S, Tamilarasan KP, Muley A, Reddy SH, Kolluru GK, Sinha S, Chatterjee S. Simulated microgravity promotes nitric oxide-supported angiogenesis via the iNOS-cGMP-PKG pathway in macrovascular endothelial cells. FEBS Lett 2010;584:3415-23.

Sung HJ, Yee A, Eskin SG, McIntire LV. Cyclic strain and motion control produce opposite oxidative responses in two human endothelial cell types. Am J Physiol Cell Physiol 2007 293:87-94.

Tinsley JH, South S, Chiasson VL, Mitchell BM. Interleukin-10 reduces inflammation, endothelial dysfunction, and blood pressure in hypertensive pregnant rats. Am J Physiol Regul Integr Comp Physiol 2010;298: R713-9.

Vaziri ND, Ni ZM, Oveisi F, Trnavsky-Hobbs DL. Effect of antioxidant Therapy on Blood Pressure and NO Synthase Expression in Hypertensive Rats. Hypertension 2000;36:957-64.

Wei Y, Whaley-Connell AT, Chen K, Habibi J, Uptergrove GM, Clark SE, Stump CS, Ferrario CM, Sowers JR. NADPH oxidase contributes to vascular inflammation, insulin resistance, and remodeling in the transgenic (mRen2) rat. Hypertension 2007; 50:384-91.

White AR, Ryoo S, Bugaj L, Attarzadeh DO, Thiyagarajan S, Chen K, Attwater S, Abbot B, Li D, Champion HC, Shoukas AA, Nyhan D, Hare JM, Berkowitz DE, Tuday EC. Early changes in vasoreactivity after simulated microgravity are due to an upregulation of the endothelium-dependent nitric oxide/cGMP pathway. Eur J Appl Physiol 2010;110:395-404.

Wilkerson MK, Muller-Delp J, Colleran PN, Delp MD. Effects of hindlimb unloading on rat cerebral, splenic, and mesenteric resistance artery morphology. J Appl Physiol 1999;87:2115-21.

Wilkerson MK, Lesniewski LA, Golding EM, Bryan RM Jr, Amin A, Wilson E, Delp MD. Simulated microgravity enhances cerebral artery vasoconstriction and vascular resistance through endothelial nitric oxide mechanism. Am J Physiol Heart Circ Physiol 2005;288:1652-61.

Wimalasundera R, Fexby S, Regan L, Thom SA, Hughes AD. Effect of tumour necrosis factor-alpha and interleukin 1beta on endothelium-dependent relaxation in rat mesenteric resistance arteries in vitro. Br J Pharmacol 2003;138:1285-94.

Xie MJ, Zhang LF, Ma J, Cheng HW. Functional alterations in cerebrovascular K(+) and Ca(2+) channels are comparable between simulated microgravity rat and SHR. Am J Physiol Heart Circ Physiol 2005;289:1265-76.

Xie MJ, Ma YG, Gao F, Bai YG, Cheng JH, Chang YM, Yu ZB, Ma J. Activation of BKCa channel is associated with increased apoptosis of cerebrovascular smooth muscle cells in simulated microgravity rats. Am J Physiol Cell Physiol 2010;298:1489-500.

Zanetti M, d'Uscio LV, Peterson TE, Katusic ZS, O'Brien T. Analysis of superoxide anion production in tissue. Methods Mol Med 2005;108:65-72.

Zhang LF. Vascular adaptation to microgravity: what have we learned? J Appl Physiol 2001;91:2415-30.

Zhang LN, Zhang LF, Ma J. Simulated microgravity enhances vasoconstrictor responsiveness of rat basilar artery. J Appl Physiol 2001;90:2296-305.

Zhou MS, Schulman IH, Raij L. Vascular inflammation, insulin resistance, and endothelial dysfunction in salt-sensitive hypertension: role of nuclear factor kappa B activation. J Hypertens 2010;28:527-35.