Increased Level of Serum Vascular Endothelial Growth Factor by Long-Term Exposure to Hypergravity

Masanobu OSHIMA1), Hiromi SUZUKI2), Xiaoying GUO1, 3), and Hiroko OSHIMA1)

1)Division of Genetics, Cancer Research Institute, Kanazawa University, Kanazawa 920-0934, 2)Japan Space Forum, Tokyo 100-0004, Japan, and 3)School of Public Health, China Medical University, Shenyang 110001, China

Abstract: We have previously demonstrated that short-term exposure to hypergravity at 2G for 4 h induces expression of cyclooxygenase-2 (COX-2) in the mouse heart. Moreover, expression of vascular endothelial growth factor (VEGF) is also induced in the heart in a COX-2-dependent manner. Here, we demonstrate that long-term exposure of mice to 2G for 24 h resulted in a significant increase of serum VEGF level, although expression of COX-2 and VEGF in the heart decreased to the 1G-control level. Moreover, increase of serum VEGF was not suppressed by treatment with COX-2 inhibitor, indicating that VEGF was induced in a COX-2-independent manner. These results suggest that gravitational force contributes to maintenance of the serum VEGF level.

Key words: hypergravity, vascular endothelial growth factor

Astronauts experience hypergravity of 3.2G at launch and 1.4G on re-entry. They are also exposed to microgravity during space flights or residence in a space station. For space exploration in the future, it is important to know how gravitational changes affect the physiological and pathological status of the human body in order to prevent unexpected outcomes under altered gravity. To date, several animal experiments have been performed to investigate biological and biochemical responses to hypergravity and microgravity. Since opportunities for conducting animal experiments under microgravity are limited, experiments under hypergravity are important using special centrifugation apparatus (see below). The results from such hypergravity experiments are useful for predicting the effects of microgravity as well as hypergravity on the human body.

We have previously demonstrated that short-term exposure to hypergravity at 2G for 4 h [2G (4H)] induces expression of cyclooxygenase-2 (COX-2; gene symbol, Ptgs2) in the heart [7]. COX-2 is a rate-limiting enzyme for prostaglandin biosynthesis, which plays important roles in a variety of pathological responses including tumorigenesis and diabetes [5, 6, 8]. Moreover, we have shown that exposure to hypergravity for 4 h induces expression of vascular endothelial growth factor (VEGF; gene symbol, Vegfa) in the heart of wild-type mice but not in COX-2 gene knockout mice [7], suggesting that VEGF expression in the heart under...
hypergravity is dependent on COX-2 induction. However, the role of VEGF in the heart under hypergravity has not been elucidated yet.

To further investigate the role of VEGF expression under hypergravity, mice were exposed to long-term hypergravity at 2G for 24 h [2G (24H)] using the Centrifugal Acceleration Test Facility at the Japan Aerospace Exploration Agency (JAXA, Tsukuba, Japan). Centrifugation with a 7.25-m arm at 15.70 rpm produced a hypergravity condition of 2G. A cage-mounting module was attached at the end of the arm that allowed one-degree freedom, thereby ensuring that the net G field was perpendicular to the floor of the mouse cage. The behavior of the mice was monitored with a CCD camera throughout the centrifugation experiments. Temperature and moisture in the cages were maintained at 24 ± 2°C and 42 ± 2%, respectively. We used eight C57BL/6 female mice at 8 weeks of age (CLEA, Tokyo, Japan) for centrifugation. Four out of 8 mice were treated with a COX-2 inhibitor, meloxicam (Daiichi Pharmaceutical, Tokyo, Japan), at 10 mg/kg/day by oral administration from 3 days before centrifugation. The other 4 mice were used as untreated controls of the 2G (24H) exposure group. Age-matched C57BL/6 females were used as 1G controls under normal gravity. The animal experiments were carried out with approvals of the Committee on Animal Experimentation of Kanazawa University (Kanazawa, Japan) and the Japan Aerospace Exploration Agency (JAXA, Tokyo, Japan).

Immediately after stopping centrifugation, mice were anesthetized with diethylether and euthanized by collection of whole blood by heart puncture. After blood collection, half of the heart was fixed in 4% paraformaldehyde for histological examination and the other half was used for total RNA extraction using ISOGEN solution (Nippon Gene, Tokyo, Japan). Immunostaining for COX-2 was performed as previously described [7]. Rabbit anti-COX-2 polyclonal antibody (Cayman Chemical, USA) was used as the first antibody. Expressions of COX-2, COX-1 (gene symbol, Ptgs1), and VEGF were examined by RT-PCR as previously described [7]. The following primer sets were used for the respective genes: COX-2 (F-5’-CTTCCTACAGCACAGCAGATGAA-3’, R-5’-GCGTGCACATGGTTAATCGGTCTTTTC-3’); and VEGF (F-5’-CTTCCTACAGCAGAAGCAGATGAA-3’, R-5’-GCGTGCACATGGTTAATCGGTCTTTTC-3’). RNA samples prepared in previous experiments with short-term exposure to 2G for 4 h [2G (4H)] were also used in the expression analysis of this study. The band intensities of RT-PCR results were measured using Image J (NIH, USA). We also measured the serum VEGF levels of the 2G (24H)-exposed and 1G control mice using Mouse VEGF ELISA kit (RayBiotech, Norcross GA, USA).

As shown in Fig. 1A, expression of COX-2 was not found in the heart of 2G (24H) exposed mice nor that of the 1G-control mice. This result contrasts sharply with induction of COX-2 in the heart by short-term exposure to 2G (4H) (Fig. 1B, C). Consistently, COX-2 expression was not detected by immunostaining in the hearts of 2G (24H)-exposed mice nor in the hearts of 1G control mice (Fig. 2A, B). In the previous study, however, COX-2 expression was detected in vessels of 2G (4H)-exposed mouse hearts [7]. Although COX-1 is a constitutively expressed enzyme in most tissues, its expression level was significantly increased in the 2G (4H)-exposed mouse heart (Fig. 1B, C). However, we found the same amount of COX-1 mRNA level in the hearts of 2G (24H)-exposed mice as in the 1G control mice (Fig. 1A, C). Consistent with the results of COX-2 expression, VEGF was at the basal level in the 2G (24H)-exposed mouse hearts and in the 1G control mice, although its level was slightly but significantly increased in the 2G (4H)-exposed mouse hearts. Treatment with meloxicam did not affect the expression level of these genes in the 2G (24H) group. Accordingly, it is possible that COX-2, COX-1 and VEGF were transiently induced in the heart by hypergravity as an acute response, and then the expression decreased to the normal level within 24 h. It has been reported that induction of COX-2 plays an important role in protection of the heart against ischemic stress and infarction [9]. In the heart of the 2G (24H)-exposed mice, however, we did not find any ischemic changes, such as necrosis or infarction (Fig. 2C, D). These results, taken together, suggest that long-term exposure to hypergravity does not cause ischemic stress in the heart, and thus induction of COX-2 and VEGF are not required for heart function under hypergravity. Expression of COX-2 is induced not only by ischemia but also by mechanical stress, serum stimuli, and inflammatory responses. It is
Fig. 1. Representative RT-PCR analysis of COX-2, COX-1, and VEGF in the heart of 1G control, 2G (24H)-exposed, and meloxicam-treated 2G (24H)-exposed mice (A), and 1G control and 2G (4H)-exposed mice (B). GAPDH was used as an internal control. (C) Relative band intensities of RT-PCR results to that of COX-1 level at 1G is shown as mean ± SD. “Meloxicam” indicates meloxicam-treated mice. Asterisks indicate significant difference versus respective 1G control (4H). Note that expression levels of COX-2, COX-1 and VEGF are increased only in the 2G (4H)-exposed mouse heart tissues.
possible that some of these stimuli were generated temporarily in the heart by hypergravity, which resulted in transient induction of heart COX-2. However, the molecular mechanism underlying COX-2 induction under hypergravity remains to be investigated.

Interestingly, we found that the serum VEGF level was significantly increased in the 2G (24H)-exposed mice compared with the 1G control mice (Fig. 3). Although meloxicam treatment resulted in a slight decrease of the VEGF level, it was not a significant reduction. These results indicate that long-term exposure to hypergravity induces VEGF expression in tissues other than the heart in a COX-2-independent manner. In this study, we did not determine tissue(s) expressing VEGF under hypergravity. However, it has been reported that increased mechanical stretch in the artery causes induction of VEGF in the vessel walls [4]. Therefore, it is possible that hemodynamic changes caused by constitutive gravitational force induced VEGF in blood vessels, increasing the serum VEGF levels. Accordingly, it is possible that increased gravitational stress affects systemic angiogenesis through induction of serum VEGF from blood vessels.

On earth, normal gravity at 1G continuously stimulates our body. Accordingly, 1G gravity may also contribute to the basal level of serum VEGF. It has been demonstrated that induction of immediate early genes in cultured cells is significantly suppressed when experiments were performed in the Space Shuttle [3], indicating that 1G gravitational stress is required for

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Fig. 2. Immunostaining for COX-2 in the heart of 1G-control (A) and 2G (24H)-exposed (B) mice. COX-2 expression was not detected in the 2G (24H)-exposed mouse heart including vessels nor in the 1G control. Histology of the heart (H&E) of 1G control (C) and 2G (24H)-exposed (D) mice. Note that neither necrosis nor infarction were found in 2G (24H)-exposed mouse heart. Bars in A, B, and C, D indicate 100 µm and 200 µm, respectively.
induction of these genes. Therefore, our results suggest the possibility that the serum VEGF level is decreased by long-term exposure to microgravity, like in a space station. Gene targeting of VEGF in the mouse leads to severe reduction in the size and caliber of the developing blood vessels [1, 2]. Accordingly, further experiments under microgravity are required to investigate the serum VEGF level and vascular development by breeding mice in a space station.

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**References**