Possible Utilization of Gerbils for Spaceflight Experiment

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Abstract: Effects of 14 days of hindlimb suspension on the body composition and hematological and muscular properties were studied in 9-wk old male Mongolian gerbils (*meriones unguiculatus*). Hemoglobin concentration and hematocrit levels were increased after hindlimb suspension. White blood cell and platelet counts were lowered, on the contrary. Significant atrophy was noted in soleus and adductor longus muscles, although the weights of other hindlimb muscles were stable. The mass of other organs, such as brain, heart, liver, kidney, and spleen, were not influenced by hindlimb suspension, either. Even though the daily food intake was similar to the controls, water consumption was very low during hindlimb suspension. This phenomenon may be the major cause for the hemoconcentration. However, the lowered platelet counts may suggest that increased blood viscosity and/or possibility of clot formation may be modified by activation and/or consumption of platelets. The unloading-related water loss from plantaris muscle was less than other species. These phenomena may be closely related to the greater tolerance to dehydration in this animal. It was suggested that *meriones unguiculatus* could be one of the unique animals to be used for spaceflight experiment.

Key words: *meriones unguiculatus*, hindlimb suspension, responses of muscle and blood

It is well-reported that the removal of weight-bearing activity from the hindlimbs induces a drastic effect on the muscles responsible for maintenance of posture and ground support. Pronounced atrophy, particularly of the slow-twitch soleus muscle, has been a consistent finding in response to gravitational unloading (J. Appl. Physiol. 73: 51-57, 1992; J. Appl. Physiol. 81: 145-151, 1996). It was also reported that the unloading-related fiber atrophy and shift of phenotype in soleus muscle of Wistar Hannover rats were prominent relative to those of other species (Am. J. Physiol. Cell Physiol. 290: C981-C989, 2006). Hindlimb unloading by tail suspension of rats causes a shift of body fluid toward head and water content of hindlimb muscles decreases (Jpn. J. Physiol. 52: 235-245, 2002). Similar phenomena are also noted in astronauts in space. Thus, it is speculated that lowered water content, dehydration, could be one of the factors for the unloading related changes in muscle properties. However, it is not known how dehydration influences the muscle plasticity. Therefore, effects of hindlimb suspension in *meriones unguiculatus* that is known to be highly tolerant to dehydration were studied in the present study.

Methods

All experimental procedures were conducted in accordance with the Japanese and American Physiological Society *Guide for the Care and Use of Laboratory Animals*. The study was also approved by the Animal Use Committee at Osaka University.

Animals were separated into 3 groups randomly; pre-experiment control, hindlimb unloaded group, and the age-matched controls (n=5 in each group). The experimental group was anesthetized with sodium pentobarbital (5 mg/100g body weight, *i.p.*). Then, a sticky tape (~5 mm width and 3 cm length) with good cushion was placed longitudinally on the dorsal and ventral sides of the mid-tail. These tapes were further surrounded cross-sectionally by a tape. Such treatment was performed loosely in order to keep the blood flow intact. A string was inserted through the gap between the tail and tape and fastened to the roof of cage at a height allowing the forelimbs to support the weight, yet prevent the hindlimbs from touching the floor or the wall of the cage. The animals could reach the food and water freely by using their forelimbs. Solid food and water were supplied *ad libitum*. But the daily intakes of food and water were checked daily. The hindlimb suspension was performed for 14 days.

Samplings of venous blood and various tissues including hindlimb muscles were performed under anesthesia with *i.p.* injection of sodium pentobarbital before and after hindlimb suspension. Venous blood was withdrawn from the jugular vein using an anticoagulant, heparin-coated, syringe for determination of hematological properties, such as hemoglobin

concentration, hematocrit level, white blood cell counts, and platelet counts. Various tissues and organs, including hindlimb muscles, brain, and internal organs, were also sampled and wet weighed immediately, and then frozen for further analyses.

The soleus muscle was pinned on a cork at an optimum length and frozen in isopentane cooled with liquid nitrogen. The mid portion of the muscle was then mounted perpendicularly on a cork by using OCT (optimum cutting temperature) compound (Miles, Elkhart, IN). Cross-section of the muscle (10-μm thickness) was cut in a cryostat maintained at –20 °C. Laminin was stained by using laminin antibody and fluorescein isothiocyanate. The stained images were detected by a confocal laser scanning microscopy and then incorporated into a computer and fiber cross-sectional area was analyzed by Scion image (Scion corp.).

Water content was determined in plantaris muscle. The wet weight of whole muscle was measured on a pre-dried and weighed aluminum foil. The muscle and foil were transferred to an incubator, kept at 50 ^{o}C. The weight was checked a couple of times during 4 days. Since the stable weights were obtained after 3-4 days, the lightest weight was utilized as the dry weight. And the percentage of water content in whole muscle was calculated.

Statistical analyses: All values were expressed as means ± SEM. Significant differences were examined by ANOVA followed by Scheffé's post hoc test. Differences were considered significant at the 0.05 level of confidence.

Results and Discussion

Significant atrophy was noted in soleus and its fibers and adductor longus muscles, although the weights of other hindlimb muscles were stable. The mass of other organs, such as brain, heart, liver, kidney, and spleen, were not influenced by hindlimb suspension, either. The unloading-related water loss from plantaris muscle was less than other species (Jpn. J. Physiol. 52: 235-245, 2002). Hemoglobin concentration and hematocrit levels were increased suggesting an induction of hemoconcentration. Platelet and white blood cell counts were lowered, on the contrary. Even though the daily food intake was similar to the control, water consumption was very low during the hindlimb suspension. This phenomenon may be the major cause for the hemoconcentration. However, the lowered platelet counts may suggest that increased blood viscosity and/or possibility of clot formation may be modified by activation and/or consumption of platelets. These phenomena may be closely related to the greater tolerance to dehydration in this animal. It was suggested that *meriones unguiculatus* could be one of the unique animals to be used for spaceflight experiment.

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