Image Analysis of Bone Changes in *Hyla japonica* Exposed to Microgravity on the MIR Orbital Station

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Introduction

It is known that changes in gravity affect both the growth and metabolism of bones (Globus, et al., 1984 and Noda and Sato, 1987). The recent finding of osteoporosis in astronauts after long-term space flight is attracting close attention (Good and Rambaut, 1985). For future progress to occur space development, it is now essential to identify changes in human bones under microgravity and to establish a method for their diagnosis. Therefore, we recently studied changes in the bones of frogs (FRIS) (FRIS experiment group, 1991) exposed to microgravity for one week by image engineering analysis.

Materials and Methods

Bone changes were visualized by super enlargement radiography imaging, using a computed radiograph (CR, Fuji Photo Film Co.) (Kashima, et al., 1988) and a micro-focus tube (MFT, Type P70-II, Pony Atomic Industry Co.). In addition, bone changes were quantitatively analyzed by using a bio-imaging analyzer (BAS, Fuji Photo Film Co.) (Kashima, et al, 1989) for comparison with the visual evaluation. In a preliminary study, enlarged radiographs of bones from various sites in the body were taken. This preliminary study disclosed that the spongy bone of the spine could be clearly visualized. Therefore, the spinal bone was selected for the main study, which consisted of the following steps.

- (1) Direct 10-fold X-ray radiography, using an MFT (focus: $10 \times 10 \mu\text{m}$) and imaging plate (IP, HR type Fuji Photo Film Co.) (Amemiya, 1988)

- (2) Indirect 2-fold interpolation photographing with CR to yield a 20-fold super enlargement CR images.
- (3) Visual evaluation of the image obtained in (2) for bone changes.
- (4) Preparation of a IP (Fuji Photo Film Co.) for quantitative analysis of bone changes disclosed by (3).
- (5) Quantitative analysis of bone changes using an IP and BAS.

Results

1. The combination of a MFT and CR yielded 20-fold enlargement CR images of the frog bones.
2. Visual evaluation revealed the most marked bone changes in the caudal articulation vertebra (Fig.1).
3. The spongy bone in the caudal articulation vertebra was more dense in the control frogs than in the FRIS.
4. The average photo stimulated luminescence (PSL) level in the caudal articulation vertebra was about $10 \times$ higher in FRIS than in the controls (Fig.2)
5. Analysis of the frequency distribution of the quantum level in the posterior joint of the 7th vertebra, the distribution for FRIS was about 20% higher than that for controls (Fig.3).

Received October 12, 1991.

Presented at the 8th Space Utilization Symposium, July 1991.

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Figure 1 Super enlarged CR images of the caudal articulation vertebra of FRIS (right) and control (left).

Figure 2 The average PSL level in FRIS and controls.

Figure 3 Frequency distribution curve of the quantum level in the 7th vertebra

Discussion

Bone is composed of periosteum, cortex and medullary space. The spongy bone, which constitutes the medullary space, is visualized as linear or reticulated bone trabecula on X-ray radiograms. Bone tissue constantly undergoes resorption and formation. Spongy bone is particularly sensitive to changes in bone mineral contents resulting on changes in bone following a one week exposure to microgravity were visualized by super enlargement radiography. A quantitative analysis of the changes in the 7th vertebra disclosed an approximately 20% bone decalcification (resorption) in FRIS as compared to controls. The resorption pattern observed was similar to the pattern of age-related bone changes revealed in our previous study of Down's syndrome (Kashima, et al., 1990). Long-term exposure to microgravity seems to precipitate bone senescens.

Conclusion

Calcium is found not only in bone but also in cells, vessels, and nerves.

For this reason, it is important for future space development to reduce the risk of a reduction in calcium during a long-term space flight. In the future, we will further evaluate the relationship between gravity and bone changes to clinically establish a method for diagnosing microgravity associated bone changes.

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