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2. Research Term
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3. Research Fields
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4. Research Categories
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5. Research Theme
Effects of osteoblasts and osteoclasts under micro-gravity: Analysis of bone metabolism using fish scales

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8. Summary of Research

Background
Fish scale is a calcified tissue that contains osteoblasts, osteoclasts, and bone matrix, all of which are similar to those found in human bone. Recently, we developed a new in vitro model system using goldfish scale. This system can be used to simultaneously detect the activities of both scale osteoclasts and osteoblasts with tartrate-resistant acid phosphatase and alkaline phosphatase as the respective markers and precisely analyze the co-relationship between osteoblasts and osteoclasts. In addition, we made high-bone-turnover scales and osteoclast-rich scales as models for bone diseases during space flight. Using these scales, the following results were obtained.

Results
1) Response to the microgravity of 3D clinostat, ultrasound (US) stimulation, and several degrees of acceleration in osteoblasts and osteoclasts of goldfish scale
At 6 and 24 h after incubation under the microgravity of a 3D clinostat, we found that the osteoblastic activity decreased, while the osteoclastic activity increased. Therefore, osteoblasts and osteoclasts in the scale sensitively responded to microgravity of 3D clinostat, and the scale showed similar conditions to osteoporosis during space flight.

The osteoblastic activity significantly increased by pulsed low-intensity US (1 MHz, 60 mW/ cm² ISATA, 50 % duty factor at 0.5 Hz, 180 pulses) in 18 h of incubation at 15°C after US treatment but not in shorter incubation periods, whereas the osteoclastic activity did not change in the same incubation period. To examine the mechanism of US in osteoblasts, the insulin-like growth factor-I (IGF-I) and estrogen receptor (ER) mRNA expressions in the cultured scales were analyzed by RT-PCR. IGF-I mRNA expression increased in 3 h of incubation at 15°C after US treatment. On the other hand, ER mRNA expression was found to be higher in the US-treated scales than in the control scales in 18 h of
incubation at 15°C after treatment, although ER mRNA expression did not change in 3 h of incubation. Therefore, IGF-I mRNA expression responded more rapidly than ER mRNA expression to US, and IGF-I may have an important function in the activation of osteoblasts by US treatment.

The bone metabolism under various degrees of acceleration (0.5-, 1-, 2-, 4-, and 6-G) with a G-load apparatus was examined. After loading for 5 and 10 min, the scales were incubated for 6 and 24 h. The osteoblastic and osteoclastic activities were then measured. The osteoblastic activities gradually increased corresponding to 1-G to 6-G acceleration. In addition, ER mRNA expression was the highest under 6-G acceleration. On the other hand, the osteoclastic activity decreased at 24 h of incubation under low acceleration (0.5- and 1-G). This change coincided with tartrate-resistant acid phosphatase mRNA expression. Under 2-G acceleration, the strength of suppression in osteoclastic activity was the highest. At both 6 and 24 h of incubation, the osteoclastic activity decreased under 2-G acceleration. The strength of the inhibitory action under 4- and 6-G acceleration was lower than that under 2-G acceleration.

Since the scale osteoblasts and osteoclasts sensitively responded to physical stress, as described above, we strongly believe that the scale is a suitable model for the analysis of bone metabolism under microgravity during space flight.

2) Effects of US stimulation on high-bone-turnover scale and osteoclast-rich scale

We found that both osteoblastic and osteoclastic activities increased in the remaining ontogenic scales on the left side after the removal of all scales on the right side. Using these high-bone-turnover scales, we examined the effects of US stimulation on osteoblasts and osteoclasts under the same conditions described above. In 18 hrs of incubation at 15°C after US treatment, the osteoblastic activity increased by US treatment in the scale. Furthermore, we indicated that the osteoclastic activity in the high-bone-turnover scale decreased as a result of US treatment.

By intramuscular autotransplantation of the scale of goldfish, multi-nucleated osteoclasts (an active type of osteoclast) were observed. The scale with activated osteoclasts might be a model of bone resorption under microgravity. In this scale, osteoclastic activity decreased at 24 h of incubation after US stimulation, although osteoblastic activity did not change by US treatment.

These results suggest that US stimulation is a useful method for the cure of bone resorption under microgravity in humans.

3) Effects of novel indole derivatives on osteoblasts and osteoclasts

To develop a drug for the cure of bone diseases, the effects of melatonin, 2-bromomelatonin, 2,4,6-tribromomelatonin, and novel bromomelatonin derivatives (1-allyl-2,4,6-tribromomelatonin, 2,4,6-tribromo-1-propargylmelatonin, 1-benzyl-2,4,6-tribromomelatonin, and 2,4,6,7-tetrabromomelatonin) on osteoblasts and osteoclasts were examined using goldfish scale. All bromomelatonin derivatives, as well as melatonin, had an inhibitory action in osteoclasts. Particularly, 1-benzyl-2,4,6-tribromomelatonin possessed a stronger activity than melatonin. This chemical (10^{-10} M) still suppressed osteoclastic activity after 6 h of incubation. In osteoblasts, all bromomelatonin derivatives had a promotional action of osteoblasts, although melatonin inhibited osteoblastic activity. For example, 1-benzyl-2,4,6-tribromomelatonin (10^{9} to 10^{6} M) activated osteoblasts at 6 h of incubation, while melatonin (10^{8} to 10^{6} M) suppressed osteoblastic activity in the same incubation time.

Therefore, we strongly believe that these novel melatonin derivatives have potential for use as curative drugs for bone diseases that develop during space flight.

9. Publication List

Meeting of the Japan Society of Sonochemistry, 3-4 (2006)


10.URL