

## SUPPRESSION OF BONE RESORPTION BY BISPHTHONATE FOLLOWING INTRAMUSCULAR ECTOPIC BONE FORMATION INDUCED BY rhBMP-2

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**INTRODUCTION:** Autogenous bone, allograft and artificial bone substitutes are frequently used in orthopaedic, plastic and reconstructive surgery. Autogenous bone graft has the greatest potential for bone conduction and induction with no risk of microorganism transmission and immune reaction. However, in some cases, autogenous graft is not adequate for large defect reconstruction. Bone graft availability and donor site morbidity are other concerns. In our previous study [1], generation of intramuscular autogenous bone tissue was achieved by using recombinant human bone morphogenetic protein-2 (rhBMP-2) with  $\beta$ -tricalcium phosphate ( $\beta$ TCP) as a carrier. However, absorption occurred early after bone induction through normal bone remodeling mechanism. This study was conducted to investigate whether simultaneous administration of bisphosphonate would enable the maintenance of induced bone tissue.

**METHODS:** Eighty 12-week-old female Sprague-Dawley rats were used. Experiment 1: Single administration of rhBMP-2 (17 $\mu$ g/30 $\mu$ l) solution was applied to the  $\beta$ TCP disc 5mm in diameter and 2mm in height. The disc was inoculated into the quadriceps of rats. Specimens were harvested at 1, 2, 3, 4 weeks (n=5 in each group). Experiment 2: Simultaneous administration of Bisphosphonate (Minodronate YM529) with rhBMP-2 (17 $\mu$ g/17 $\mu$ l) solution at different YM529 concentrations (10<sup>-4</sup>, 10<sup>-5</sup>, 10<sup>-6</sup>, 0M/13 $\mu$ l) was applied to the  $\beta$ TCP disc. The discs were implanted similarly to the previous experiment. Specimens were harvested at 2 and 4 weeks (n=5 for each concentration). The specimens were soft X-rayed, H&E and TRAP (spell out) specific stained. Ratio of induced bone area to the total area of  $\beta$ TCP disc was measured by Scion image (NIH image). The number of TRAP positive cells was counted at 2-4 visual fields of each sample, and then averaged. Compressive strength test was performed on the samples at 4 weeks in Experiment 2 specimens. Plain  $\beta$ TCP discs without any drug addition of were used as controls.

**RESULTS:** Experiment 1: The ratio of bone tissue area and the number of TRAP-positive cells reached maximum after 2 weeks and then declined.

Experiment 2: The percentage of bone tissue area was well preserved even at 4 weeks (Fig.1).

Addition of YM529 decreased the number of TRAP-positive cells after 2 weeks of inoculation (Fig.2). We also observed increased bone tissue strength in the groups of 10<sup>-4</sup>M and 10<sup>-5</sup>M YM529 concentrations.

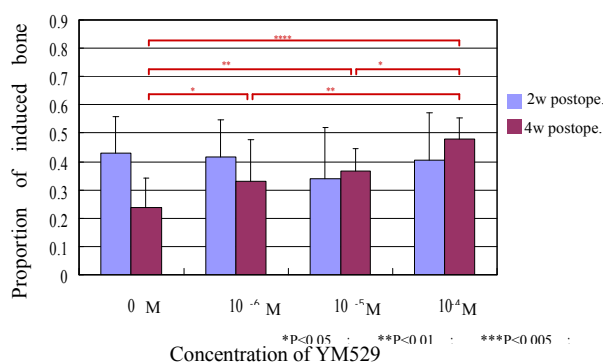


Fig. 1: Experiment 2: Simultaneous administration of YM529. Induced bone area.

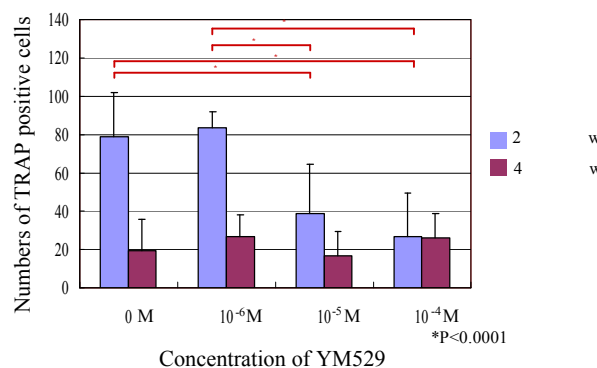


Fig. 2: Experiment 2: Simultaneous administration of YM529. Number of TRAP positive cells.

**DISCUSSION & CONCLUSIONS:** The concurrent use of bisphosphonate prevented bone absorption attributed to osteoclastic activity inhibition after bone induction by rhBMP-2. The compressive strength also increased without rapid bone absorption in the newly induced bone. The combination of rhBMP-2 with bisphosphonate in  $\beta$ TCP may have potential in clinical use for bone tissue transplantation.

**REFERENCES:** <sup>1</sup> S. Jingushi, K. Urabe, et al (2002) *J Orthop Sci* 7:490-494.