SUPPRESSION OF BONE RESORPTION BY BISPHOSPHONATE FOLLOWING INTRAMUSCULAR ECTOPIC BONE FORMATION INDUCED BY RHBM-2 –IN VIVO BONE BANKING FOR MUSCLE-PEDICLE AUTOGRFT

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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. An ideal graft bone is auto-bone tissue, of which there is an ample supply with the required form and with vascularity. Our strategy is to generate intramuscular autogenous bone tissue by using recombinant human bone morphogenetic protein-2 (rhBMP-2) with β-tricalcium phosphate (βTCP) as a carrier, and transplant as muscle-pedicle autograft. However, in our previous study (1), 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, and whether the induced bone can be used for muscle-pedicle bone graft.

MATERIALS AND METHODS:
Experiment 1&2 were conducted to investigate whether simultaneous administration of bisphosphonate would enable the maintenance of induced intramuscular ectopic bone tissue. 12-week-old female Sprague-Dawley rats were used. Experiment 1: single administration of rhBMP-2 (17µg/17µl) solution was applied to the βTCP disc 5mm in diameter and 2mm in height. The disc was inoculated into the quadriceps of rat. Specimens were harvested at 1, 2, 3, 4 weeks (n=5). Experiment 2: simultaneous administration of bisphosphonate (Minodronate YM529) with rhBMP-2 (17µg/17µl) solution at different YM529 concentrations (0, 10^{-4}, 10^{-6}, 0M/13µl) was applied to the βTCP disc. The disc was implanted similarly to the previous experiment. Specimens were harvested at 2 and 4 weeks (n=5). The specimens were soft X-rayed, H&E and tartrate-resistant acid phosphatase (TRAP) specific stained. Ratio of induced bone area to the total area of βTCP disc was measured. 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 βTCP discs without any drug addition were used as controls. Experiment 3 was to investigate whether the bisphosphonate application will decline the absorption of rhBMP-2 induced ectopic bone to normal bone tissue. RhBMP-2 (17µg/17µl) solution with different YM529 solutions (10^{-4}, 10^{-5}, 0M/13µl) was applied to the βTCP disc and inoculated as previously experiment. Four weeks after inoculation, β-TCP disc with induced bone was incised from muscle and attached to the exposed parietal bone under periosteum. Syngeneic bone obtained from iliac was managed in the same shape as β-TCP disc, then attached to parietal bone. Harvest was performed at two weeks and six weeks (n=5) after transplantation. The specimens were soft X-rayed, H&E and tartrate-resistant acid phosphatase (TRAP) specific stained.

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: Radiological features showed increased radiolucency in YM529 0M samples at four weeks compared to that of two weeks, while YM529 10^{-4}M samples showed higher density after four weeks compared to that of two weeks. The percentage of bone tissue area was well preserved even after 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^{-4}M and 10^{-6}M YM529 concentrations. In experiment 3, almost all the grafts showed bone union with parietal bone after 6 weeks of transplantation. There is no difference between YM529 0M and 10^{-4}M samples (Fig.4). In experiment 4, bone formation and bone union to normal bone were detected in both YM529 treated muscle-pedicle auto-graft (Fig.5).

DISCUSSION:
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 bisphosphonate application didn’t inhibit the bone union of induced bone to normal bone tissue. The combination of rhBMP-2 with bisphosphonate in βTCP may have potential in clinical use for muscle-pedicle auto-bone graft.

**Fig.1: Experiment 2: Simultaneous administration of YM529. Ratio of induced bone area.**

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

**Fig.3: Experiment 2: Simultaneous administration of YM529. Compression strength test. Cont.: β-TCP without any management.**

**Fig.4: Bone union score**

**Fig.5: Radiological features of muscle pedicled graft. 10^{-4}M YM529 solution treated sample; four weeks after transplantation.**

REFERENCES: