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The effect of calcitonin on increasing the effectiveness of hydroxyapatite and β-tricalcium phosphate in bone regeneration

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ABSTRACT

Introduction: Osteon® is an alloplastic material containing 70% hydroxyapatite and 30% β-TCP. Calcitonin, a hormone produced by the thyroid gland, not only prevents bone resorption by reducing both the number and the activity of osteoclasts, but also stimulates bone formation. Considering the favorable effects of calcitonin on bone formation, we hypothesized that the addition of calcitonin to Osteon would result in better bone regeneration.

Materials and Methods: To test this hypothesis, full-thickness bone defects were created bilaterally in the femoral condyles of 12 New Zealand white rabbits. The defect was filled with Osteon on one side (the control group); while on the other side, the defect was filled with a combination of Osteon and calcitonin (the experimental group).

Result: These findings showed that the number of osteoblasts and the degree of calcification in the experimental group were significantly higher than the control group (P = 0.05).

Conclusion: On the basis of these findings, it is plausible to suggest that the addition of calcitonin to hydroxyapatite and β-TCP as alloplastic materials could contribute to a greater degree of osteogenesis.

Keywords: Calcitonin, Alloplastic Bone, Bone Defects, Hydroxyapatite, Rabbit, Tricalcium Phosphate

Introduction

In many clinical situations, such as dental implant surgery, bone graft materials are required for filling bone defects or augmenting the alveolar process. Although autogenous bone is considered the gold standard for grafting in bone surgery, there is an increasing trend toward using alloplastic materials among surgeons. This is partly due to the fact that alloplastic materials are biologically acceptable and allow bone ingrowth and bone remodeling while maintaining volume [1]. These alloplastic materials have several advantages over autogenous bone, such as ample supply and lack of donor site morbidity. An ideal bone graft should fulfill the following requirements: (a) maintaining space for an optimal period of time to achieve bone ingrowth; (b) promoting osteoconduction of the neighboring cells to form bone within the graft material; (c) remodeling itself into long-lasting bone; and (d) having a predictable success rate [2, 3].

Hydroxyapatite is the major mineral component in bone; therefore, synthetic hydroxyapatite has become a common and reliable osteoconductive replacement material for bone defects [4]. Synthetic calcium phosphate ceramics, such as beta-tricalcium phosphate (β-TCP) and hydroxyapatite, are commonly used in the form of blocks, cements, pastes, powders, or granules as alternatives to autogenous bone, xenograft, or allograft materials [5]. Many of the current commercially available alloplastic materials such as Bioresorb®, Chronos®, Ceros®, Cerasorb®, and Vitoss® contain β-TCP ceramics, whereas other commercially available alloplastic materials such as PepGen P-15®, Cerabone®, Ostim®, BioOss®, and Tutoplast® contain hydroxyapatite ceramics.

Osteon® is an alloplastic material containing 70% hydroxyapatite and 30% β-TCP. The architecture of Osteon is similar to the human cancellous bone, with the

interconnected porosity and pore size capable of providing space for bone cell ingrowth [6]. Two types of Osteon grafting materials are commercially available with a particle size of either 0.5–1.0 mm or 1.0–2.0 mm. The β-TCP component of Osteon is gradually resorbed during the process of bone regeneration while the hydroxyapatite component acts as nonresorbable scaffold.

Calcitonin, a polypeptide hormone produced by the thyroid gland, plays a major role in reducing blood calcium (Ca^{2+}) level and opposing the effects of parathyroid hormone (PTH). Calcitonin prevents bone resorption by reducing both the number and the activity of osteoclasts, and also stimulates bone formation [7, 8]. Therefore, in theory, calcitonin seems to be a favorable agent in bone grafting procedures by stimulating bone formation and preventing bone resorption.

Alloplastic materials, such as Osteon, have been shown to be suitable grafting materials for bone regeneration. Considering the favorable effects of calcitonin in bone formation, it is plausible to hypothesize that the addition of calcitonin to alloplastic materials would provide better results in terms of bone regeneration. The hypothesis was tested by conduction of an animal study as described below.

Material and Methods

For this experiment, 12 white male New Zeland rabbits with an average weight of 3 kg each were used. The study protocol was reviewed and approved by the Ethics Committee of Tehran University of Medical Sciences, Iran. The animals were divided into 3 intervention groups of 1 week, 4 weeks, and 12 weeks.

The animals were anaesthetized with 2% xylazine (8 mg/kg) and 10% ketamine (50 mg/kg). A 5 cm longitudinal incision was made on the skin overlying the area of the left and right lateral femoral condyles, followed by plane-by-plane muscle dissection and incision of the periosteum. On each side, a full-thickness bone defect of approximately 4 mm in diameter and 5 mm in depth was created in the lateral femoral condyle with an orthopedic bur activated by a micromotor. The bone defect was drilled under continuous saline cooling. On one side, the defect was filled with Osteon (the control group); while on the other side, the defect was filled with a combination of Osteon and calcitonin (the experimental group). The particle size of Osteon was 0.5–1.0 mm. The Osteon of the experimental group was soaked in 1 ml of calcitonin for 15 minutes before implantation. In addition, the surgical wound was sutured in multiple layers in the end surgery.

The animals were kept caged freely, were given their usual regimen of food and water after the surgery, and were observed for signs of pain and infection. They were randomly divided into 3 groups, each comprising of 4 animals. All 4 animals of each group were sacrificed by an overdose of thiopental sodium 1week (Group 1), 4 weeks (Group 2), and 12 weeks (Group 3) into the surgery. From each animal, the implantation sites of both femurs were removed, immediately placed in 10% formalin solution, and sent to a laboratory for histological and histomorphometric analysis.

All specimens were fixed in 10% formalin for 24 hours and decalcified in 8% nitric acid for 24 hours. The tissue were then treated with lithium carbonate for 1 hour, embedded in paraffin, and sectioned to a thickness of 5.0 μm. Sections were then stained with hematoxylin-eosin and evaluated using a light microscope. All specimens were analyzed by the same histologist who was blind to the type of the graft material used in each specimen.

Results

After 1 week, the number of osteoblasts and the degree of calcification in the experimental group were significantly higher than the control group (P = 0.05); while the inflammatory cells were significantly more abundant in the control group compared to the experimental group (P = 0.05) (Figure 1).

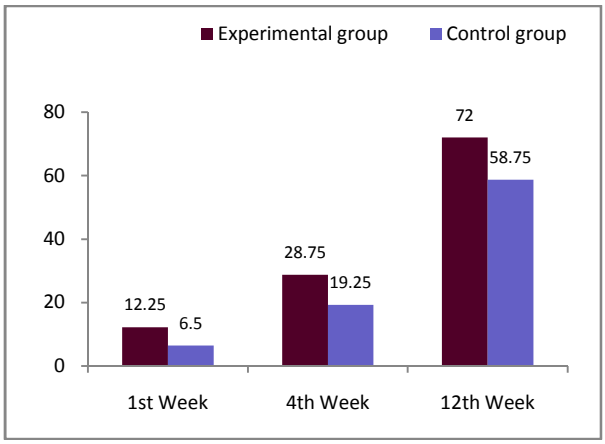

Figure 1. The percentage of calcification in the experimental and control groups

After 4 weeks, the number of osteoblasts and the degree of calcification in the experimental group were still significantly higher than the control group (P = 0.05). However, no statistically significant difference was observed between the two groups in terms of the number of inflammatory cells. The same results were observed in samples taken 12 weeks after surgery.

Discussion

The main goal of this study was to investigate the effectiveness of Osteon (control group) versus a combination of Osteon and calcitonin (experimental group) in bone regeneration. The results showed that approximately 38% of the bone defect was calcified in the experimental group; while only 30% of the bone defect was calcified in the control group. The difference between the two groups in terms of the mean percentage of calcification

was statistically significant. Furthermore, the number of osteoblasts in the experimental group was significantly greater than the control group.

Kim et al. evaluated the use of Osteon as a sinus graft material in human subjects and measured the effects of healing 4 and 6 months after surgery [6]. Having used Osteon for sinus graft, bone specimens were collected from lateral sinus using a trephine bur 4 or 6 months after surgery. Histology of the bone specimens was prepared to indicate the suitability of the materials and the successful healing of the graft. Bone biopsy indicated more lamellar bone 6 months after surgery compared to the present study. There was an increased accumulation of newly formed bone and resorption of graft materials during the healing process. In general, these observations suggest that Osteon is a suitable material for grafting purposes and allows normal healing. These findings are consistent with the results of our animal study showing Osteon alone to have positive effects in bone regeneration. Furthermore, we have demonstrated that the addition of calcitonin to Osteon can contribute to a greater degree of osteogenesis.

Conclusion

Alloplastic materials are commonly used to fill different types of bone defects in various maxillofacial structures, including defects around dental implants. Hydroxyapatite and β-TCP are among the most frequently used alloplastic materials for these purposes. Our findings demonstrate that the addition of calcitonin to hydroxyapatite and β-TCP gives rise to a greater degree of osteogenesis.

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Conflict of Interest: 'None declared'.

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