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Boosting effect of bisphosphonates on osteoconductive materials: a histologic *in vivo* evaluation

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Background and Objective: The effect on bone regeneration, of adding pamidronate disodium to bovine-derived hydroxyapatite, was histologically evaluated, using the sheep bone model.

Material and Methods: Twenty-four intrabony defects were prepared in the lower jaw of eight sheep using trephine 6 mm burs. One cavity was left unfilled and the other two were filled with bovine-derived hydroxyapatite (BioOss™) alone (control group) or with bovine-derived hydroxyapatite mixed with pamidronate disodium (Aredia) (case group), respectively. After 6 wk, the animals were killed and the coded samples observed using an optical microscope. The percentage of regenerated bone, number of osteoclasts and amount of inflammation was recorded. Statistical analysis was carried out using chi-square and Mann–Whitney *U*-tests.

Results: The results manifested a significant difference in the amount of bone formation, with the most being observed in the case group and the least in the negative-control group ($p < 0.001$). Significantly fewer osteoclasts were observed in the case group than in the other groups ($p < 0.001$). The amount of inflammation did not seem to differ within the case and control groups ($p > 0.05$).

Conclusion: Adding pamidronate disodium to bovine-derived hydroxyapatite improves its osteoconductive and regenerative specifications. Further study should determine the systemic effects of a single local administration of these drugs, and their appropriate dose and type, with minimal risk.

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Bisphosphonates are a group of drugs that have an inhibitory effect on osteoclasts. It is thought that while the osteoclast activity in bone is inhibited, the continued activation of osteoblasts may lead to bone regeneration and new bone formation. Pamidronate disodium is an injectable nitrogen-containing bisphosphonate (1).

Long-term prescription of bisphosphonates has been widely reported to be useful in the treatment of bone-related pathologic conditions, such as osteogenesis imperfecta (2–4), osteoporosis (5,6), spondiloarthropathy (7), bone loss after allogenic stem cell transplantation (8), bone fracture in pediatric patients on chronic steroid therapy (9), bone metastasis or pain caused by it (10,11), calcification in elderly cancerous patients (breast and

prostate carcinoma and multiple myeloma) (12), acute vitamin D intoxication (13) and Paget disease (1). Bisphosphonates have also been used to help improve the osseointegration of implants (14–16) or to improve alveolar bone resorption in peri-implantitis (17), although these applications have been questioned by others (18).

Recently, many reports have questioned the safety of long-term systemic administration of bisphosphonates and have reported a possible side-effect, named osteonecrosis (which is similar to osteoradionecrosis), in the oral region (19–24). Terms such as bisphosphonate-related osteonecrosis or bisphossy jaw have been suggested to describe this condition, the cause of which is not yet clear (25). These concerns suggest that extreme caution should be taken in prescribing these drugs.

Local delivery of drugs by a degradable carrier has the potential to achieve high local drug levels and avoids systemic toxicity. Intravenous access, renal function monitoring, and subsequent surgical removal may not be required when degradable local delivery modalities are used. The primary benefit achieved with local delivery vehicles is the ability to obtain high levels of local medicament without increasing systemic toxicity. Medicament-loaded bone cement represents the current standard as an antibiotic delivery vehicle, and biodegradable alternatives to medicament-loaded bone cement are also being used clinically (26–28). These facts suggest that the side-effects related to long-term systemic use of bisphosphonates may be minimal or absent when bisphosphonates are used topically, which needs to be further investigated.

Different forms of hydroxyapatite, alone or in combination with other products, have been tested by many authors as a carrier of different medicaments, mostly antibiotics, especially in the treatment of local bone infections, such as osteomyelitis (29–39).

In view of the positive effects of bisphosphonates on the bone-regeneration process, the authors decided to evaluate histologically the local effects of a single local administration of pamidronate disodium mixed with bovine-derived hydroxyapatite on the bone regeneration process, using the sheep bone model.

Material and methods

In this experimental animal study, eight sheep, less than 1 yr of age and weighing approximately 35 kg, were used. The ethics committee of Hamedan School of Dentistry (Hamedan, Iran) and the National Animal Care Society (Tehran, Iran) approved the protocol of this study. Based on standards approved by Tehran Veterinary School (40) (Tehran, Iran), the animals were prepared for surgery and sedated with an intramuscular injection of xylazine hydrochloride 2% (0.1 mg/kg). A mandibular block injection was performed with 2 to 5 ml of lidocaine in order to obtain complete anesthesia. A crestal flap was then prepared in the edentulous region of the mandible on both sides, and full-thickness flaps were reflected with the aid of an elevator. After irrigating the area with normal saline solution, three intrabony defects were prepared using a 6-mm-diameter trephine bur, starting 3 mm anterior to the first molar tooth, each with 2 mm distance from the border of the last hole. The depth of each hole was decided to be 6 mm. One miligram of pamidronate disodium (Aredia™ 90 mg; Novartis Pharmaceutical Corporation East Hanover, NJ, USA) was dissolved in 10 ml of sterile distilled water and mixed with 1 g of 250–1000 μm particles of powdered bovine-derived hydroxyapatite (BioOss® Spongiosa; Geistlich Pharma, Wolhusen, Switzerland). Two holes were randomly filled with either bovine-derived hydroxyapatite alone (positive-control group) or with the mixture of bovine-derived hydroxyapatite and pamidronate disodium (case group), and the third defect was left empty as the negative-control group. The flaps were then closed with silk sutures. In general, 24 defects were prepared with the same procedure. The animals were given an appropriate diet and attended to in terms of their oral hygiene, in addition to a routine checkup of the operation site taking place for up to 6 wk after the operation, when the animals were killed using the vital perfusion technique with formalin. The samples were then retrieved using cutting disks.

In order to decalcify the blocks, samples were floated in formic acid 10% for 15 d, after which the blocks were thoroughly rinsed with water. The specimens were then dehydrated in an ascending series of alcohol rinses and, in order to increase their translucency, they were placed in 50% and 100% solutions of methyl salicylate for 2 and 5 h, respectively. The blocks were then embedded in paraffin. Ten transverse cross-sections with the diameter of 5 μm were made through each defect, using a microtome device (Jung, Frankfurt, Germany). The samples were then stained with hematoxylin and eosin and were observed with an optical microscope (Nikon Eclipse E400; Nikon, Sumida-Ku, Japan), linked through a digital camera (Nikon Fujix HC_300Zi; Nikon) to a personal computer. The images were captured using a histometry software with image-capturing capabilities (IMAGE-PRO PLUS 4.5; Media Cybernetics, Milano, Italy). To avoid possible bias, coded samples were used. The histomorphometry was carried out with the aid of the histometry software. The percentage of regenerated bone was recorded by the pathologist. In order to estimate the osteoclast activity, the number of giant cells was recorded. The level of local inflammation was also recorded by the pathologist.

Statistical analysis was carried out with chi-square and Mann–Whitney *U*-tests. All the values were expressed as mean ± standard deviation. Statistically significant differences were set at $p < 0.05$.

Results

Six weeks after surgery, the case and positive-control cavities presented an optimum clinical healing, while the healing in the negative-control group was clinically incomplete. Histologically, the negative-control cavities were filled with fibrovascular connective

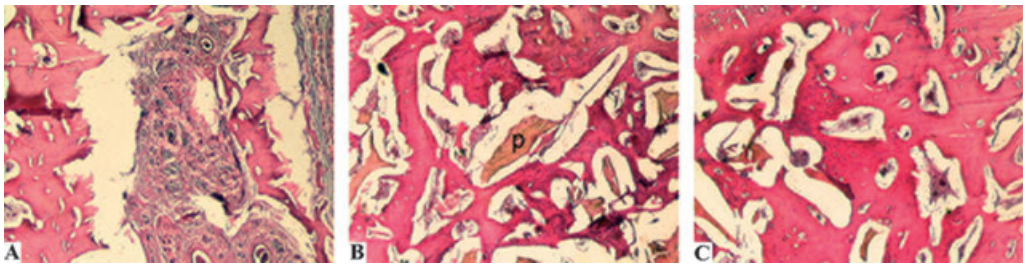

Fig. 1. Histologic view of different cavities. (A) Negative-control group: areas of fibrovascular connective tissue surrounded by bony trabeculae are visible. (B) Control group: matrix particles (p) are seen within generated bone trabeculae. (C) Case group: the matrix particles are densely embedded in the bone trabeculae (hematoxylin and eosin staining, ×10 magnification, optical microscope).

tissue surrounded by bone trabeculae. Few scattered inflammatory cells were still visible in the regenerated tissues of this group of defects (Fig. 1A).

The positive-control defects, which were filled only with inorganic bovine hydroxyapatite, demonstrated trabeculae formation around implant particles. The formed trabeculae loosely surrounded the matrix particles. Inflammatory cells were not identifiable in the cavity area (Fig. 1B).

In the cavities of the case group, which were filled with bovine-derived hydroxyapatite mixed with pamidronate disodium, bone trabeculae were densely formed around the implant particles and these particles seemed to be embedded in the regenerated bone. The diameter of bone trabeculae was higher than in the control group. Like the control group, no inflammatory cells were visible in the observed regions (Fig. 1C).

As seen in Fig. 2, there was a significant difference among the three groups regarding the amount of regenerated bone. The case group had the highest amount of bone regeneration, while the negative-control group had the least ($p < 0.001$, chi-square and Mann–Whitney *U*-tests). As seen in Fig. 3, the case group had the fewest osteoclasts ($p < 0.001$), while the positive- and negative-control groups had a similar number ($p > 0.05$). The level of inflammation was also significantly different between the negative-control group and the case- and positive-control groups ($p < 0.001$), while the case and positive-control groups showed no significant difference ($p > 0.05$).

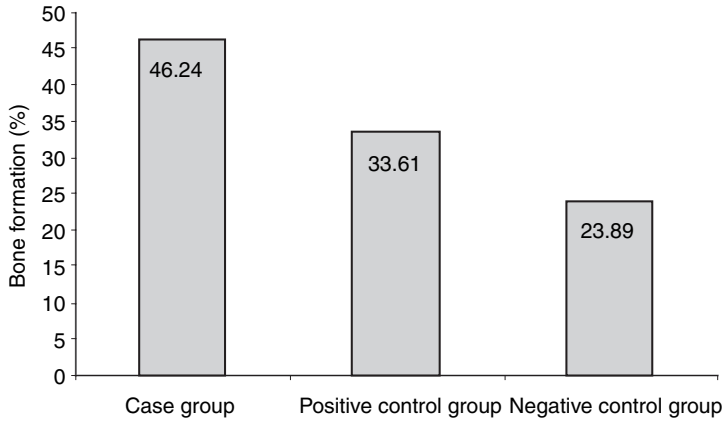

Fig. 2. Comparison of bone formation (%) in different groups. A significant difference in bone formation between the three groups was determined, according to chi-square and Mann–Whitney *U*-tests ($p < 0.001$).

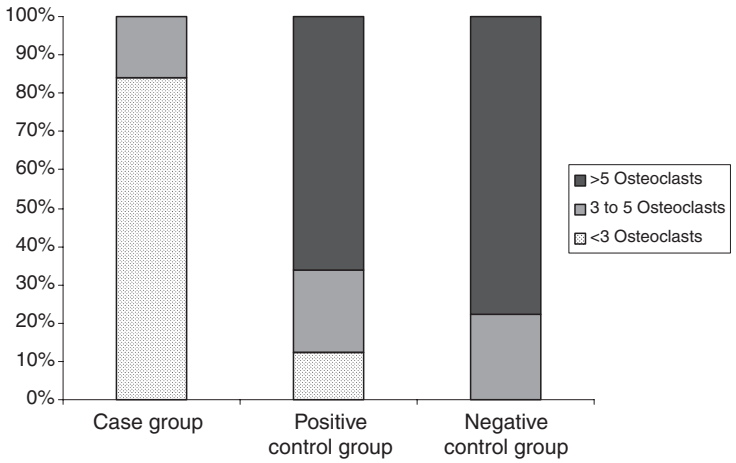

Fig. 3. Comparison of osteoclast count in 100 μm^2 for each group. The number of osteoclasts in the case group differed significantly from the control groups, according to chi-square and Mann–Whitney *U*-tests ($p < 0.001$).

Discussion

The aim of this study was to evaluate the short-term effects of a single local administration of pamidronate disodium, without considering its possible long-term side-effects, distribution pattern, biologic half-life, etc. The authors tried to examine the topical effects of this drug histologically on the

bone regeneration process to establish whether it could be used as a means to accelerate bone healing after surgical procedures with bone involvement in the oral cavity. Although local delivery of bisphosphonates has been reported to involve minimum systemic manifestations (26–28), as several reports have related long-term systemic use of these drugs with osteonecrosis (19–25) and there is a possibility that these drugs may remain in the site and leach into the system for a long period of time, the authors suggest that further studies should be carried out to determine how long the drug remains in the site, its appropriate dose and the systemic effect of local application. According to the current concerns over the long-term effects of bisphosphonates and the potential for osteonecrosis, any use of these drugs should still be considered with extreme caution.

The strong difference between the case and control groups of this study showed that pamidronate disodium improved bone formation by increasing the percentage of bone trabeculae and decreasing the number of osteoclasts in the regenerated bone. These findings support other studies, suggesting the relevance of this group of drugs for the treatment of several bone-related pathologic conditions (1–17).

In this study, bovine-derived hydroxyapatite was used as the local carrier of bisphosphonates. This material was selected according to several other reports that have suggested hydroxyapatite as a suitable drug carrier and also as an osteoconducting material (29–39). The results of this study also confirmed these specifications for bovine-derived hydroxyapatite.

The results obtained also proved that pamidronate disodium improved the bone healing process by boosting the regeneration process as a result of reducing the number of osteoclasts and increasing the amount of trabeculae in the regenerated tissue. These findings support other studies that have reported the same features of this material on the bone-regeneration process (41–43).

Several techniques have been introduced to impregnate a local carrier with a medicament. In the present study, hydroxyapatite powder was simply mixed with the pamidronate disodium solution. This technique has been previously reported for powdered hydroxyapatite and an antibiotic (30,31). The dose used to mix the materials was experimentally determined according to the absorbance capacity of the hydroxyapatite granules, tested previously in the laboratory. There have been reports of mixing hydroxyapatite with similar amounts of antibiotics, which was also used as a guideline (30). In conclusion, enhancing bovine-derived hydroxyapatite with pamidronate disodium improves its osteoconductive and regenerative specifications. The authors suggest further studies to evaluate the possible long-term systemic effects of a single local administration of bisphosphonates. The appropriate dose for local administration, as well as the pattern of its local and systemic distribution, should also be determined. The optimum type of bisphosphonates and the most suitable carrier for these purposes should also be tested and identified.

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