Journal of Dental Biomaterials
Shiraz University of Medical Sciences, Shiraz, Iran

Journal of Dental Biomaterials. 2015;**2**(3)

Original Article

Effect of Combined Calcium Hydroxide and Accelerated Portland Cement on Bone Formation and Soft Tissue Healing in Dog Bone Lesions

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ARTICLE INFO

Article History
Received 12 April 2015
Accepted 21 July 2015

Key words:
Bone substitutes
Accelerated portland cement
Calcium hydroxide
Bone formation
Wound dehiscence

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Abstract

Statement of Problem: Recent literatures show that accelerated Portland cement (APC) and calcium hydroxide Ca $(OH)_2$ may have the potential to promote the bone regeneration. However, certain clinical studies reveal consistency of Ca $(OH)_2$ as one of the practical drawbacks of the material when used alone. To overcome such inconvenience, the combination of the Ca $(OH)_2$ with a bone replacement material could offer a convenient solution.

Objectives: To evaluate the soft tissue healing and bone regeneration in the periodontal intrabony osseous defects using accelerated Portland cement (APC) in combination with calcium hydroxide Ca $(OH)_2$, as a filling material.

Materials and Methods: Five healthy adult mongrel dogs aged 2-3 years old (approximately 20 kg in weight) with intact dentition and healthy periodontium were selected for this study. Two one-wall defects in both mesial and distal aspects of the 3rd premolars of both sides of the mandible were created. Therefore, four defects were prepared in each dog. Three defects in each dog were randomly filled with one of the following materials: APC alone, APC mixed with Ca $(OH)_2$, and Ca $(OH)_2$ alone. The fourth defect was left empty (control). Upon clinical examination of the sutured sites, the amount of dehiscence from the adjacent tooth was measured after two and eight weeks, using a periodontal probe mesiodistally. For histometric analysis, the degree of new bone formation was estimated at the end of the eighth postoperative week, by a differential point-counting method. The percentage of the defect volume occupied by new osteoid or trabecular bone was recorded.

Results: Measurement of wound dehiscence during the second week revealed that all five APCs had an exposure of 1-2 mm and at the end of the study all samples showed 3-4 mm exposure across the surface of the graft material, whereas the Ca $(OH)_2$, control, and APC + Ca $(OH)_2$ groups did not show any exposure at the end of the eighth week of the study. The most amount of bone formation was observed in APC group which was significantly different with all other groups ($p < 0.05$).

Conclusions: Despite acceptable soft tissue response of Ca $(OH)_2$, this additive material could not be suggested because of negative effects on bone formation results.

Cite this article as: Khorshidi H, Raoofi S, Sabagh S, Behboud Z, Mozafari Gh, Ashraf MJ. Effect of Combined Calcium Hydroxide and Accelerated Portland Cement on Bone Formation and Soft Tissue Healing in Dog Bone Lesions. J Dent Biomater, 2015; 2(3): 97-102.

Introduction

The use of substitute material for bone graft in intraosseous bone lesions has been well established. Among the various methods and materials used, clinicians prefer those that are cheaper, have easy application, and yield acceptable results. There are currently many studies on mineral material as bone substitute in periodontal regeneration [1]. The relative cheapness of mineral material and their easy mass production are among the factors that contribute to their increased evaluation and investigation.

Calcium hydroxide Ca $(OH)_2$ has been used to induce apexification in the pulpless teeth with incomplete root formation [2]. This material demonstrates antibacterial properties [3], enhances tissue dissolution, and induces bone formation [4]. Studies suggest that a rise in pH induced by calcium hydroxide combined with the availability of $Ca2^+$ and OH ions has a stimulating effect on enzymatic pathways and therefore mineralization. However, the interactive role of calcium hydroxide with cytokines and growth factors in the stimulation of an osseous response is unclear [5].

Results of basic research and clinical studies have proven the effect of an oily Calcium Hydroxide suspension (OCHS) on the bone regeneration in closed defects subsequent to periapical surgeries, in bone cysts and post-extraction alveoli [6]. Its osteostimulative effect seems to rely on many factors, as the deposit action of the Calcium Hydroxide which sustains the bone metabolism in a constant, mild alkaline environment; the stimulation of the angiogenetic bone growth with concentration of the growth factors next to the defect wall; and the reduction of the inflammation in the operated site, which enhances the wound healing. However, from clinical point of view, some practical problems could arise when using the OCHS alone; the material has a low consistency and, therefore, cannot ensure a sufficient stability of the mucoperiosteal flap, especially in one-wall and circular defects. Frequently, a collapse of the mucoperiosteal flap cannot be avoided, followed by the reduction of the space necessary for the regeneration process [7].

To overcome such inconvenience, the combination of the OCHS with a bone replacement material could offer a convenient solution. By this approach, the chemical and biological properties of the OCHS could be combined with the mechanical properties of the bone replacement material. In this combination, the OCHS could enhance the bone and the periodontal healing, while the bone replacement material could avoid the collapse of the mucoperiosteal flaps and ensure the post-surgical stability of the wound. Therefore good biological properties of Ca $(OH)_2$ in combination with the mechanical properties of the bone replacement material can enhance the periodontal healing [8].

Portland cement is being widely used as a construction material and there is currently evidence for its clinical use [9]. In 1993, mineral trioxide aggregate (MTA) was introduced to dentistry. Mineral trioxide aggregate (MTA) is a powder aggregate, containing mineral oxides [10]. Besides its non-cytotoxicity [11], it has good biological action [12] and stimulates the repair [13] because it allows cellular adhesion, growth and proliferation on its surface [14]. MTA consistently allows for the overgrowth of cementum, and it may facilitate the regeneration of the periodontal ligament and formation of the bone [15].

The main composition in MTA is Portland cement [16]. Previous studies have shown that MTA and Portland cement have similar antimicrobial effect [17], cytotoxicity, and biocompatibility [18].Therefore, since Portland cement is less expensive than MTA, it seems that it can be used as a suitable substitute for MTA as a filling material. By adding calcium chloride to Portland cement, one of its main problems which is delayed setting time is solved [19]. Some researchers suggest using accelerated Portland cement (APC) for orthopedic purposes because added calcium chloride has no adverse effect on osteoconductive characteristics of Portland cement [20]. The proliferation of the connective tissue and growth towards the apical epithelium is prevented after using Portland cement for intraosseous lesions in dogs [21] .

However, it is currently unknown how calcium hydroxide may influence bone healing when used in conjunction with APC.

We aimed to assess the effect of Ca $(OH)_2$, APC, and a combination of both on bone regeneration and soft tissue response in dog bone lesions.

Materials and Methods

Animal selection, management, surgical protocol and preparation followed the requirements of the Ethics Committee routines of Experimental Animal Research Center at Shiraz University of Medical Sciences.

This experimental study was performed on five healthy adult mongrel dogs aged 2-3 years old (approximately 20 kg in weight) with intact dentition and healthy periodontium.

To prepare APC, 0.3g calcium chloride (Kimia Material Co, Iran) was added to 3 g of Portland cement type 2 (Fars Cement Co, Iran). To make a mixture of APC + Ca $(OH)_2$ (Kimia Material Co, Iran) , the same proportion of each one was used. Three forms of graft material were sterilized through the exposure to continuous UV rays for 24 hours.

Surgery was performed under general anesthesia induced by an intravenous sodium pentobarbital (20 mg/kg), and maintained on halothane gas (2%) in 50% oxygen. In addition, local anesthesia was induced by 2% lidocaine injection.

During the initial phase of surgery, the 2^{nd} and 4^{th} premolar teeth of both sides of the mandible were extracted. After two months and in the second phase of surgery, the graft materials were used. Under general and local anesthesia, a sulcular incision was made from the mesial of the first premolar to the mesial part of the first molar passing the crest of the edentulous region on both sides of the mandible and the flap was elevated. Two one-wall defects were created in both mesial and distal aspects of the 3rd premolars of both sides of the mandible with a depth and width of 4mm. Therefore four defects were prepared in each dog. Three of the defects in each dog were randomly filled with one of the following materials: APC alone, APC mixed with Ca $(OH)_2$, Ca $(OH)_2$ alone and the fourth defect was left empty (control). The flaps were repositioned and sutured at pre-surgery position with resorbable chromic 2-0 cut gut.

Upon the clinical examination of the suture site, post-operative restoration of the amount of dehiscence from the adjacent tooth was measured after two and eight weeks, using a periodontal probe mesiodistally. Since the mesiodistal width of the created and filled lesion was 4 mm, an opening of 4 mm was considered as 100% exposure.

The animals were euthanized 8 weeks following the surgical procedure by an intravenous injection of concentrated sodium pentobarbital. Block sections including the surgical sites were removed at sacrifice. The sections were rinsed in sterile saline and fixed in 10% formalin for 1 week. After rinsing in water, the sections were decalcified in 10% nitric acid for 1 week and embedded in paraffin. Serial sections, 5 µm thick, were cut in a mesial–distal direction at intervals of 60 µm. The six most central sections from each block were stained with hematoxylin and eosin (H&E) and examined using light microscopy.

For histometric analysis, the degree of the new bone formation was estimated at the end of the eighth postoperative week, by a differential point-counting method, using an integration eyepiece with 100 equidistant points. A total of 500 points were counted in six histological sections (final magnification X100), the percentage of points lying on connective tissue and bone trabeculae are proportional to their volume density. The percentage of the defect volume occupied by new osteoid or trabecular bone was recorded.

Statistical analysis

All quantitative data were expressed as the mean and standard deviation. Statistical analysis was performed through two-way ANOVA and Tukey's -HSD test using SPSS software. A value of $p < 0.05$ was considered statistically significant.

Results

At the second week following the surgery, all five APCs had an exposure of 1-2 mm and at the end of the study, all samples showed 3-4 mm exposure across the surface of the graft material and the hardened cement was palpable with a periodontal probe from the exposure site. The Ca $(OH)_2$, control, and APC + Ca $(OH)_2$ groups did not show any exposure at the end of the eighth week of the study. The mean percentage and standard deviation of the defect volume occupied by new osteoid or trabecular bone is shown in Table 1.

The most amount of bone formation was observed in APC group. The APC group showed a significant

Table 1: The mean percentage and standard deviation (SD) of the defect volume occupied with bone

Groups	Mean	SD
APC	80%	7%
APC+$Ca(OH)_2$	44%	5%
Ca(OH)2	35%	4%
Control	53%	4%

difference with all other groups (p < 0.05). The least amount of bone formation was observed in Ca $(OH)_2$ group. The Ca $(OH)_2$ group showed a significant difference with all other groups (p < 0.05). The amount of bone formation in the control group was significantly more than APC + Ca $(OH)_2$ groups and Ca $(OH)_2$ group (p < 0.05). The APC + Ca $(OH)_2$ groups showed significantly more bone formation in comparison to Ca $(OH)_2$ groups (p = 0.001).

Discussion

Various treatments such as the use of guided tissue regeneration alone or in combination with different types of bone grafts, root surface demineralization, enamel matrix derivative, or the application of growth factors have been employed with varying degrees of success in the periodontal regeneration and bone healing process [22].

There is increasing interest to use minerals to replace the bone graft. The main characteristic of the minerals used to replace the bone graft is to fill the empty cavity and maintain this space. These minerals are absorbed by the host and replaced with bone; therefore, the bone regeneration in the periodontal bone lesions is accelerated without filling the cavity by the soft tissue. The main purpose of the present study was to assess the bone formation and soft tissue covering APC graft through changes in chemical composition by adding Ca $(OH)_2$.

An eight week healing interval has been considered useful to study the periodontal regeneration in dog models. In addition, Choi *et al*. reported that no differences in the bone regeneration were noted between 8 and 24 week intervals. Therefore, an 8-week healing period is sufficient to observe the initial healing process. Kim *et al*. reported that one- and three-wall intrabony defects appear to be reproducible models to evaluate candidate technologies for periodontal regeneration. Critical size of the periodontal intrabony defects in dogs has not been defined; however, the size of 4×4 mm was used in similar studies [23,24].

Our results showed suitable healing of the soft tissue in the presence of Ca $(OH)_2$ alone as a bone substitute material. In fact, adding Ca $(OH)_2$ to Portland cement led to improvement of the soft tissue repairing appropriately. Some authors have emphasized the positive effect of calcium hydroxide on bone formation [25,26]. Ito and colleagues found that Ca $(OH)_2$ might accelerate bone induction [27]. One related hypothesis is that Ca $(OH)_2$ might induce bone formation by increasing the expression of the gene for bone sialoprotein, a protein expressed in mineralizing tissue [28].

However, in the present study, the addition of $Ca(OH)_2$ did not improve the bone regeneration with an emphasis on osteoid formation and new trabecular bone, while the results of bone regeneration without adding Ca $(OH)_2$ was better. Consistently, in a randomized controlled trial comparing the effect of the oily form of calcium hydroxide suspension in combination with open flap debridement and open flap debridement alone for treating infra-bony periodontal defects, the researchers found no significant effect or superior clinical outcomes when calcium hydroxide was added [29].

Inconsistent results about the effect of Ca $(OH)_2$ in different studies could be attributed to the difference in the concentrations used for Ca $(OH)_2$. Recently, in an in vitro study, it was shown that higher concentrations of oily calcium hydroxide suspension was cytotoxic and inhibited bone formation, whereas moderate concentration of Ca $(OH)_2$ shows more positive results [30]. Moreover, from clinical point of view, some practical problems could arise when using OCHs alone; the material has a low consistency and, therefore, cannot ensure a sufficient stability of the mucoperiosteal flap, especially in one-wall defects. Frequently, a collapse of the mucoperiosteal flap cannot be avoided, followed by the reduction of the space necessary for the regeneration process [6,7].

So, the negative effect of Ca $(OH)_2$ on bone formation in our study may be due to faster washing of Ca $(OH)_2$ as a graft material and also inability to maintain the space for the time it takes for bone formation.

The result of the present study showed using APC alone significantly promotes bone formation in comparison with the control group. This osteogenic effect of APC was also observed in Dokami *et al*.'s study. Through a histological analysis, they evaluated the effect of APC as a bone graft substitute on experimentally-created three-walled intra-bony defects in dogs. Their histopathological observation showed a significant increase in bone regeneration following the implantation of APC compared with the controls [21].

Similar results were also reported by Ca´ssio *et al.* evaluating bone repair using MTA in bone defects. There was a statistically significant difference between the groups with inserted MTA and the control ($p < 0.05$). Results showed that the use of MTA could induce the bone regeneration.

It is believed that the deposition of hard tissue over the MTA is related to features such as good sealing ability, biocompatibility, alkaline pH, the presence of calcium and phosphate ions in its formulation, the capacity to attract blastic cells, promotion of a favorable environment for cementum formation [31], osseous and cementum conductive effect [32], the stimulus to adhesion and cell proliferation [33], the stimulus to expression of alkaline phosphatase by fibroblasts [34], and osteocalcin and other interleukins by osteoblasts [35].

Despite the bone formation improvement, soft tissue healing was not suitable through APC, and complete wound dehiscence was observed at the end of the eighth week after the surgery in all defects filled by APC. Although APC has been shown to improve bone regeneration, it seems that its combination with $Ca(OH)_2$ is not promising even though the tissue response might be favorable.

Conclusions

Despite acceptable soft tissue healing, due to the negative effect of adding Ca $(OH)_2$ on bone reconstruction including osteoid formation and new trabecular bone, and the better results of bone reconstruction without adding Ca $(OH)_2$, the authors do not recommend this additive material.

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