Efficiency of Three-Calcium Phosphate Cements in Combination with Bio-Glass for Tibia Bone Recovery in Dogs; Based on Histo-Morphological Evaluations

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Abstract: The aim of this study was to investigate three-calcium phosphate (TCP) and mg contented bio-glass efficiency for healing of tibia bone defects in dogs as an animal model. Six male dogs (25-29 kg) were used. Animals were grouped; 1) dogs were treated for three months and 2) dogs were treated for six months that each group were included three dogs. Four holes (a, b, c and d) with 5mm diameter were established by dental trephine on bone body as experimental defects. Hole (a) was without any treatment (control), hole (b) were filled with TCP, hole (c) were filled with TCP (75%) and mg included bio-glass (25%) and hole (d) were filled with TCP (60%) and mg included bio-glass (40%). At day-30 and -90, samples of two groups were taken for histo-morphological observations. Fibrous connective tissue and osteogenesis in woven appearance along with section of formed natural bone were histo-morphological signs of hole (a) at day-30 and -90. Also, in this sample a hole with 763836 micron area with osteogenesis and woven connective tissue were observed. While, bone with haversian system in environment of hole (b) were clear. In center of bone, a hole with 423996 micron with bone marrow cells was observed. In its environment lamellar bone with numerous haversian system were identified. In hole (c), osteogenesis in woven form with fibrous and 223996 micron area hole were observed. Also in its environment, narrow newly formed bone was seen. In hole (d), a lot of newly formed woven-like bones along with natural bone were observed. The hole was in 174774 micron area with woven bone and considerable connective tissue, without inflammatory reactions. It was concluded that three-calcium phosphate (TCP) in combination with mg contented bio-glass is so effective material for healing of tibia defects.

Key words: Tibia • Three-calcium phosphate • Bio-glass • Osteogenesis

INTRODUCTION

Bone healing is one of the most critical functions of body. Occurred trauma cause soft tissues laceration, damaging and hemorrhage at bone environment that results in blood vessels (thrombus) creation in defected bone and its environment [1]. Further progress in bone recovery occurred via callus formation. If broken bone pieces were locating in front to front position, bone healing is occur quickly and without external callus formation [1]. Specific synthetic materials can be a replacement for bone materials. These include natural coral, hydroxyapatite, three calcium phosphate (TCP), bioactive glasses and synthetic polymers that are using in experiments on animal model as filler for bone disorders [2]. TCP is a compound with application in ceramic industry. It has not structural protein and has biological adaptation without inflammation induction in body and because of its slow absorption, doesn’t change plasma Ca and P concentration. TCP acts as a trellis for bone growth. Bone growth rate is depends on graft site, bone specify,
osteoblast cells and graft fixation. Therefore when TCP is located in outside of periosteum, bone growth is minor. Biological absorption of TCP occurred by passive solution and absorption via osteoclasts [3, 4]. Absorption rate of TCP depends on chemical structure, bone density and graft contacted surface. Biological absorption rate for TCP is considerable more than hydroxy apatite (one of bone cements) [3, 4]. Bio-glasses as stimulatory product in osteogenesis is without side effects and is a suitable selection for combination with TCP cement [5]. Optimum hollows for bone recovery experiments by TCP are 100-500 micrometer for obtaining more absorption rate and better biomechanics characterizes [3, 4].

The aim of this study was to investigate three-calcium phosphate cement efficiency for healing of tibia bone defects induced by artificial hollowing in dog as an animal model, from the clinical and histo-morphological points of view.

MATERIALS AND METHODS

Six head male dogs (20-25 Kg) were randomly selected for this experiment. Animals were kept in similar nutritional and environmental conditions. Next, dogs were randomly divided into two experimental groups. TCP were obtained by calcium carbonate (Merck, Germany) and CaHPO4 (Merck, Germany) due to solid phase reaction. TCP were mixed with bio-glass gel in three different combinations; 90%TCP: 10%Bio-glass, 75%TCP: 25%Bio-glass, 60%TCP: 40%Bio-glass.

Prepared sample disks (5mm diameter and 3 mm height) were pressed under 200 mega Pascal pressure. Next they were heated (1000-1200°C) for three hours [6]. 12 hours before surgery animals were in fasting (without feeding) and 4 hours without water intake.

Anesthesia was done by IV-injection of 19 mg sodium thiopental. After anesthesia, the inside of tibia bone was scraped and was washed.

Surgical Procedure: 10 cm in length paralleled with tibia bone length were cut. Fascia and endodermal tissues were separated, hereby diaphysis and metaphysis were observed. Four holes (a,b,c and d) with 5cm diameter were established by dental trephine on bone body. Next, medullary canal were detected and observed with application orthopedic drill.

TCP tablets were sterilized by X-ray (25 KGY, for 10 hours).

Post-Surgical Care: Antibiotics (including penicillin and gentamycin) were IV-injected daily. Stitches were removed at 12-day post-surgery. Clinical symptoms were daily examined to stitches removal time and after that were examined weekly for three months. Whole samples from defected (experimental) segment of tibia were taken follow dogs fatality for histo-pathological investigations. The samples were placed in 10% formalin buffer.

Preparation of Histo-Pathological Sections: After samples fixation and observation, tissues and fascia and muscles in bone environment, especially from upper side of callus were removed. After fixation, decalcification was done from bone tissue with application of 10% nitric acid. Next, papering process includes dehydration, clearness, dealcoholization, forming with paraffin and microtome has been conducted.

Finally, sample sections (5-6 micrometer diameter) were taken for staining and subsequent histo-pathological studies.

Statistical Analysis: Data obtained from observations were grouped as two experimental groups; pre- and post-treatment, furthermore, t-test was done for detection of significant differences between groups.

RESULTS

Hole1 (control) had lower osteogenesis as compare with hole2, at end of 1 month treatment period. In microscopic observations, fibrous tissue with homogenous pink to red appearance like lacing port were observed in damage zone, that bone immature spicules were visible. In group2 spicules were mineralized and had osteoid like appearance.

Active fibroblasts, many inflammatory mono nucleic macrophages and blood vessels have been observed. After one month experiment a big part of cement were not observed. The cement was surrounded with inflammatory mono nucleic macrophages.

In group1 hole had 784532 micron area that surrounded with bone and mature tissue with minor blood vessels and inflammatory signs were significantly less. Also, signs of spicule maturation and their conversion to lamellar bone have been observed. Fatty and active osteoblast surrounded tissue. In group1 after animal keeping for three months, so many formatted osteoblasts were observed.
According to Figure 1, in this group spicule extent, number and diameter was more than group1 (control). In hole2 (Figures 2 and 3), damage zone in bone was covered with netted bulk that after one month, reddish narrow osteogenic spicules and subsequently some vessels have been observed. At the left and upper sides of image and signs of an osteogenic spicule with active osteoblasts in its environment and in button, signs of monocytes or macrophages can be seen. But in section of this hole taken from 3-month experiment (TCP), large part of TCP has been observed and surrounded with mature spicules. Formatted osteogenic blades had haversian system and large extent, diameter and cement replacement better than condition observed in Figure 8. At right and button of Figure 3, many small purple granules were observed. In hole 3, a section were prepared from one month old samples (TCP 75% and Mg bio-glass) showed overall osteogenesis and cement replacement was better than pervious condition (at the beginning of experiment). At right and button, there are so many purple granules in the cement (H&E, 284X)
DISCUSSION

TCP cement is new generation bone substitutes, with potential clinical applications in orthopedics. TCP is one of the best materials for bone repair, because of their biocompatibility and osteoconductivity. The cement can be shaped into any complicated geometry or filled into any intricate cavity, within its setting period. It can adapt to the bone cavity, offering a good fixation and optimum tissue biomaterial contact necessary for stimulating bone in growth [7].

Senaha et al. [8] showed potential of bio-glasses in providing long-lasting fixation of implants to bone under weight-bearing conditions in dogs. The most notable outcome of the histological analysis is the replacement of TCP by new bone, as the healing process progresses. This is the osteoconductive property, an ideal requirement for a bone substitute which provides better strength to the healing site [9, 10] but, it seems resorption of calcium phosphate cement takes a relatively long time [11].

Mousavi et al. [12] showed that the mixture of collagen type I and calcium phosphate bone cement is a good choice for the healing of segmental bone defects and provides a more rapid regeneration of bone defects in compare with control or groups without collagen. In current experiment it was noted that TCP in combination with mg- contended bio-glass is a good situation for bone cement preparation for bone defects recovery in dog model, too (Figures 3-8).

Findings of this experiment about efficiency of TCP combination with biomaterials (such as bio-glass) for acceleration of bone defect recovery is according to Mousavi et al. [12] in rabbits and Ozturk et al. [11] in rat model. Currently, Lee et al. [13] in a study on dogs reported that calcium phosphate glass cement was replaced rapidly with an abundant volume of new bone for 4 × 4 mm 1-wall intra-bony defects. In technical mean evidences of present study exactly were in agreement with Lee et al. [13] reports.

In conclusion, three-calcium phosphate (TCP) in combination with mg contended bio-glass is so effective material for healing of tibia defects in shorter time. Also, bio-glass is a good situation for combination with TCP.
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