Biological hydroxyapatite as bone graft substitute; a preliminary report

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ABSTRACT
The biological calcium hydroxyapatite was isoplated and purified from local cow bones in laboratories of the University of Mosul. The chemical analysis of the product shows that the purity of the biological calcium hydroxyapatite is 99%. The biological calcium hydroxyapatite was prepared locally as bone graft substitute, from cheap unlimited resource. The biological calcium hydroxyapatite was implanted around induced fracture of forearm in 4 male white rabbits. The forearm fracture united thirty days after the experiment. The biological calcium hydroxyapatite was implanted inside the medullary cavity of the femur and around fracture area in another 4 male white rabbits. The implantation biological calcium hydroxyapatite was reasonable by callus around the bone and in medullary cavity of bone without any chronic inflammatory changes of foreign body reaction. We conclude that biological calcium hydroxyapatite can be used as bone graft substitute.

Key words: calcium hydroxyapatite, cow bone, bone graft substitute.

Clinical management of vascular defects often requires bone grafts. The standard treatment for autogenous bone harvested from sites such as the iliac crest. In addition to the problem of donor site morbidity and the limited supply of graft material, there is the additional operating time associated with harvesting procedures.

A bone graft substitute that can match the clinical performance of autogenous bone could alleviate these deficiencies. The limited availability and high cost of bone graft, as well as concerns of transmittable infectious disease have lead to the development of several bone graft substitutes [1-6]. There are many osteoconductive bone graft substitutes including tricalcium phosphate, coraline hydroxyapatite, calcium hydroxyapatite ceramic, porous calcium phosphate cermics, a polymeric bone substitute, calcium sulfate, bioactive...
Material and methods

The study carried out in the University of Mosul laboratories during October 2002 to March 2003. The study is a limited experimental study to isolate biological calcium hydroxyapatite from cow bone, and to implant in it induces fractures in 8 white rabbits.

A- Isolation and purification of biological calcium hydroxyapatite: The biological calcium hydroxyapatite was isolated from local cow bones by performing the following procedure:

The raw local cow bones were boiled in tap water for five hours to cook the meat that is attached to the bones and to dissolve the gelatin. The bones were treated with hot detergent solution to remove the fatty materials, washed thoroughly with water, then dried in oven at 60 °C degrees. The dried bones were crushed, ground with mortar then sifted. The resultant powder was degreased by stirring with low boiling point petroleum ether (60-80°) for one day, filtered off and dried to get white powder. To a (14 g) sample of the final powder, 100 ml of hydrochloric acid (7.5%) was added with stirring. The stirring was continued for three days. The hydroxyapatite and the other inorganic materials of the bone were dissolved, leaving the organic materials and collagen. The solid materials were filtered off and filtrate was made alkaline by adding concentrated sodium hydroxide solution until the acidity value (pH) became about 7 to obtain a white precipitate. The precipitate was filtered off, washed thoroughly with distilled water until the washing water become neutral and gave negative test about chloride ion, then dried as a white powder.

B. Experiment on laboratory animal: The dried white powder of the biological calcium hydroxyapatite was sterilized by autoclave at 120°C for 60 minutes. The powder mixed with 20 mg of gentamicin under aseptic preparation and left to try in autoclave at 50 degrees.

1. Four male New Zealand white rabbits from animal house of the College of Medicine, University of Mosul were selected, weighing 1.5, 1.6, 1.7, and 1.8 kg. The animals were anaesthetized with intramuscular ketamine hydrochloride (20 mg/kg body weight). After preparation of the skin, the right femur bones were exposed through longitudinal incision. The bone were broken by angular stress and a fragment of the biological calcium hydroxyapatite 2-3 millimeter in diameter was applied around the fracture site. The wound was closed with 5.0 vicryl, and external dressing applied as sponglage. A dose of 6000 unit of procaine penicillin was given intramuscularly, chloramphenicol to the diet of the animals according to veterinary medicine advice. X-ray was taken on the 2nd day, and 30th day.

2. Other four male New Zealand white rabbits from animal house of the College of Medicine, University of Mosul were selected, weighing 1.5, 1.2, 1.0, and 1.5 Kg. The animals were anaesthetized with intramuscular ketamine hydrochloride (20 mg/kg body weight). After preparation of the skin, the right femur was exposed through lateral longitudinal incision. The femoral bone osteotomized, the medullary canal filled with bone and the distal segments were packed with fragment of the biological calcium hydroxyapatite and fragments of the bone. To ensure that the implant remained in place, the muscles sutured to interpose between bone and soft tissues of the implant site. This was done to see fate of biological calcium hydroxyapatite when it is implanted in medullary cavity of bone. A dose of 6000 unit of procaine penicillin was given intramuscularly, chloramphenicol added to the diet of the animals according to veterinary medicine advice. Thirty days later, the four animals were killed by high dose of thiobarbitone. The sites of osteotomy were carefully exposed. The bones were removed, and fixed with 10% formaldehyde solution. After fixation, they were decalcified in 10% formic acid. The bone then dehydrated and demineralized the bone, leaving only the soft tissues and bone matrix. This was done to ensure that thin sections could be examined histologically. Thin sections embedded in paraffin wax, were cut and stained with haematoxylin and eosin.
Results

The last white product was identified by infra-red reflection spectroscopy and X-ray diffraction using monochromatic CuKα as calcium hydroxyapatite. The infra-red reflection spectrum (KBrs dis) show strong broad peak at 3660 Cm⁻¹ attributed to O-H bond stretching, a strong broad peak at 1196 Cm⁻¹ for P-O bond stretching, and medium peak at 1080 Cm⁻¹ related to P-O bond. The X-ray diffraction calculation indicates that the crystal structure of calcium hydroxyapatite is hexagonal, P6 3/m, with a = 9.441 and c = 6.904. The chemical analysis of the product shows that the purity of the product is 99%.

Result of Experiment on laboratory animals: All the animals of right forearm fracture walked normally at end of 3rd week, and clinical examination showed fracture union. The X-ray of forearm showed complete healing at the end of thirty days (Fig. 1).

The histopathological examinations of the femoral bones show that there is new bone formation in form of callus around and in medullary cavity of bone in different stages of maturation. No evidence of foreign body reaction, granulomas, abnormal giant cells or inflammatory cellular response to the implants was detected (Fig. 2, 3).

Figure (1): The upper two x-rays of forearm taken postoperatively, the lower two x-rays of forearm taken thirty days later show complete union of fractures.

Figure (2): Histological examination shows new bone formation at different stages of healing including callus and cartilaginous tissue inside the medullary cavity of femoral bone.

Figure (3): Histological examination shows new bone formation at different stages of healing without any evidence of chronic inflammation or giant cell reaction on the surface of femoral bone at fracture site.
Discussion

Calcium hydroxyapatite are non-toxic substances which provoke little reaction from the patient and may, make them, both chemical and physical, that make them suitable alternatives to bone grafts. In animal experiments, the biological compatibility of calcium hydroxyapatite to bone and bone marrow has been demonstrated by many investigations. Hydroxyapatite, synthetic and partially occurring materials, are now in use as substitutes for cancellous bone grafts. The primary usefulness is in filling bone defects in areas where bone graft thickness is not important.3,4,6

The bone substitute used in this study was prepared to be porous hydroxyapatite with very high purity reaching 99 %. The bone substitute used in this study was well accepted by the host animals, causing no ill effects or inflammation in the surrounding tissues. The implant exhibited neither toxicity, nor foreign body reaction on histological assessment.2,6

The medium number of animals used in this experiment is due to shortage of facilities during the period of study, but it is sufficient to stimulate more study in this field where financial and technical support is available.

The biological calcium hydroxyapatite used in this study could be easily prepared in any local laboratory from local unlimited resources when the technique applied strictly. The fixed size, spongiform encephalopathy transmission pathogen, bone, bone is negligible if high temperature extraction process is used, and furthermore the disease is unknown in our country and in surrounding countries. The prepared biological calcium hydroxyapatite can be prepared in form of powder or granules form.7-9

Calcium hydroxyapatite is a safe and convenient implant material which aids the regrowth of bone in defects produced by the surgical excision of benign bone tumors. Porous or granules calcium, hydroxyapatite implanted into bone defects can provide a suitable framework for human osteogenesis and compare with well others bone substitutes such as allofactors and xenografts. The biological calcium hydroxyapatite used in this study is not xenograft since the hydroxyapatite isolated chemically from the bone.6,9,10

More studies on large animals are indicated to assess the bone graft substitute function, and to assess its antibiotic carrying, and to assess its function after it's mixing with cancellous bone graft, bone marrow, or other bone graft osteoinductive bone graft substitute. We conclude primarily that biological calcium hydroxyapatite can be used as bone graft substitute, but controlled studies are needed to clarify the real advantages of this bone graft substitute.

References


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