Biological hydroxyapatite as bone graft substitute; a preliminary report

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ABSTRACT

The biological calcium hydroxyapatite was isolated 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 white male 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 ends in another 4 male white rabbits. The implanted biological calcium hydroxyapatite was replaced by callus around the bone and in medullary cavity of bone without any chronic inflammatory changes or 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.

الخلاصـة

تم استخلاص وتصفية هايدروكسي ابتايت الكالسيوم الحيوي من عظام الأبقار المحلية وذلك في مختبرات جمعة الموصل. اظهر التحليل الكيميائي أن نقاوة هايدروكسي ابتايت الكالسيوم الحيوي وصلت 99 %. تم زرع هايدروكسي ابتايت الكالسيوم الحيوي حول كسر محدث في عظام الساعد لأربعة من الأرانب البيضاء والتحمت العظام بعد ثلاثين يوما من التجربة. وتم زرع المادة داخل التجويف الداخلي لعظم الفخذ وحول نهاية العظم بعد كسر محدث لأربعة أخرى من الأرانب البيضاء. تبين أن هايدروكسي ابتايت الكالسيوم الحيوي المزروعة حول وداخل العظام استبدلت بالنسيج العظمي وبدون ظهور علامات التهابية مزمنة أو تفاعل الجسم الغريب. يمكن الاستنتاج أن هايدروكسي ابتايت الكالسيوم الحيوي يمكن أن تكون بديلا لترقيع العظام.

Clinical management of osseous defects often requires bone grafts. The standard for treatment is 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 transmitting infectious disease have lead to the development of several bone graft substitutes[1-5].

There are many osteoconductive bone graft substitutes including: tricalcium phosphate, coralline hydroxyapatite, calcium hydroxyapatite ceramic, porous calcium phosphate ceramics, a polymeric bone substitute, calcium sulfate, bioactive

bone cement (injectable calcium preparations)[2,6-13]. The osteoinductive bone graft substitute including : demineralized bone matrix, recombinant human osteogenic protein-1, Recombinant human bone morphogenetic protein-2-filled cages; synthetic bone morphogenic protein is also an effective bone graft substitute[5,14-21]. Artificial bone made of hydroxyapatite composites can be used to fill a cavity or bridge a small gap[7-9,20].

The aim of this report was to produce an effective bone graft substitute from local resources, which is easily fabricated and preserved, biocompatible with bone, and biodegradable for the treatment of bone defects.

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 it in induced 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 gm) sample of the final powder, 100ml 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 9 to obtain a white precipitate. The precipitate was filtered off, washed thoroughly with distilled water until the washing water became neutral and gave negative test about chloride ion, then dried as a white powder[23].

B. Experiment on laboratory animal: The dried white powder of the biological calcium hydroxyapatite was sterilized by autoclave at 120 °C for 90 minutes. The powder mixed with 20 mg of gentamicin under aseptic precaution and left to dry 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, weighting 1.5, 1.5, 1.6, 1.7 kg. The animals were anaesthetized by intramuscular ketamine hydrochloride (20 mg/kg body-weight). After preparation of the skin, the right forearm bones were exposed through longitudinal incision. The bones were broken by angular stress , fragment of the biological calcium hydroxyapatite 2-3 millimeter in diameter was applied around the fracture site. The wound was closed by 5-0 vicryl, and external dressing applied as splintage. A dose of 6000 unit of procaine penicillin was given intramuscularly, chloramphicol added 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, weighting 1.5, 1.6, 1.6, 1.65 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 cavities in both proximal and distal segments were packed with fragment of the biological calcium hydroxyapatite and fragment applied around the bone end. To ensure that the implant remained in place, the muscles sutured to interpose between bone ends and the muscles were apposed over 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, chloramphicol 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 osteostomy were carefully exposed. The bones were removed, and fixed with 10% formaldehyde solution. After fixation, they were decalcified in 10% foramic acid. The decalcification process 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

Result of crysallochemical characterization of biological calcium hydroxyapatite: The last white product was identified by infra-red reflection spectroscopy and X-ray diffraction using monochromatic Cukα as calcium hydroxyapatite [23]. The infra-red reflection spectrum (KBr disk) show strong broad peak at 3150 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 1060 Cm^{-1} related to P-O bond [23]. 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 animal: 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), (fig. 3).

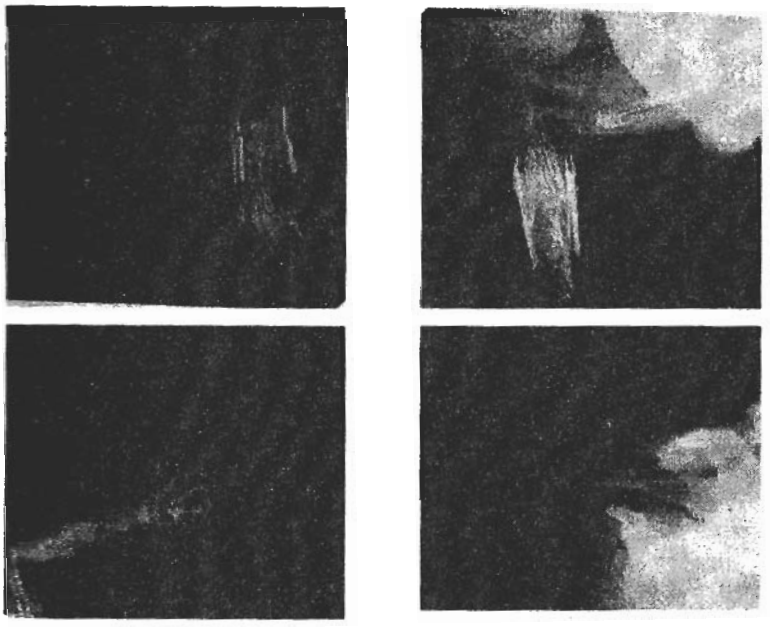

Figure (1): *The upper two x-rays of forearm taken postoperatively, the lower two x-rays of forearm taken thirty days later show complete union of fractures.*

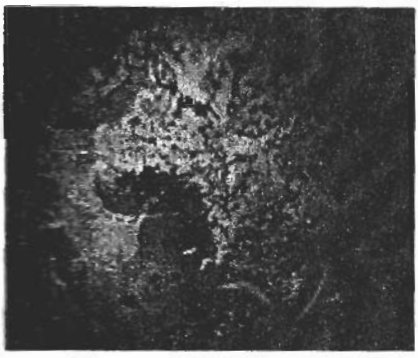

Figure (2): *Histological examination shows new bone formation at different stages of healing including callus and cartilaginous tissue inside the medullary cavity of femoral bone.*

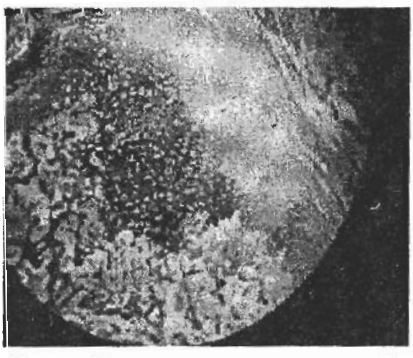

Figure (3): *Histological examination shows new bone formation at different stages of healing without any evidence of chronic inflammation or giant cell reaction on the surface of femoral bone at fracture site.*

Discussion

Calcium hydroxyapatite are non-toxic substances which provoke little reaction from tissues and have many properties, 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 investigators. Hydroxyapatite, synthetic and naturally occurring materials, are now in use as substitutes for cancellous bone grafts. The porous hydroxyapatite are invaded by blood vessels and osteogenic cells; provide a scaffold for new bone formation. Their primary usefulness is in filling bone defects in areas where graft strength is not important[2,3,4,9].

The bone substitute used in this study was proved to be calcium 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 response on histological assessment[22,23].

The small number of animals used in this experiment is due to shortage of facilities during the period of study, but we think that it is sufficient to stimulate more study in this field when 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 resource when the technique of isolation applied strictly. The risk of bovine spongiform encephalopathy transmission through bone graft material derived from bovine 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 or in form of porous ceramic[22-24].

Calcium hydroxyapatite is a safe and convenient implant material which aids the regeneration of bone in defects produced by the surgical excision of benign bone tumors. Porous or granular calcium hydroxyapatite implanted into bony defects can provide a suitable framework for human osteogenesis and compares well with other bone substitutes such as allografts and xenografts. The biological calcium hydroxyapatite used in this study is not xenograft since the hydroxyapatite isolated chemically from the bone[4,7,9,10].

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.

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