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A non-steroidal anti-inflammatory drug (ketoprofen) does not delay β-TCP bone graft healing

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ABSTRACT

β-Tricalcium phosphate (β-TCP) is a suitable biomaterial in oral and maxillofacial surgery since it can induce a rapid proliferation of woven bone. Granules, prepared by the polyurethane foam method, were implanted in critical size defects performed in the femoral condyles of New Zealand rabbits. Animals were studied after 8 and 28 days. Ketoprofen (a non-steroidal anti-inflammatory drug (NSAID)) was given for 8 and 28 days to evaluate its effects on the healing of the graft. Before euthanasia, the rabbits received an intravenous injection of fluorescent microbeads. Bones were analyzed by microcomputed tomography. β-TCP granules induced metaplastic bone trabeculae as early as 8 days post-surgery. At 28 days, the amount of bone was increased and the biomaterial volume decreased due to simultaneous macrophagic resorption. The amount of macrophages labeled with microbeads was less in the grafted area than in the vicinal intact marrow spaces. Ketoprofen had no effect on the amount of bone formed and on the number of labeled macrophages. The influence of small doses of NSAID, given in a short duration period, did not present deleterious effects on bone graft healing.

1. Introduction

Synthetic calcium/phosphate biomaterials are nowadays currently used for the repair of bone defects. They offer considerable safety compared with bone allografts or xenogenic bone. An ideal biomaterial must be able to induce a localized osteogenesis which constitutes the first step of the ossification process by a modeling process; it should be followed by a phase of resorption of the material. Tricalcium phosphate (β-TCP) has been recognized as a suitable ceramic material with bioactive properties for several decades [1–3]. β-TCP is used in maxillofacial and orofacial surgery and in dental implantology but has received little attention in orthopedics because of its friability, which precludes its use in weight-bearing areas [4–7]. Mixtures of β-TCP and hydroxyapatite (HA) have been proposed (and are often referred as BCP – biphasic calcium phosphate) [8]. However, BCP properties depend on an optimum balance between the HA phase (more stable) and the β-TCP phase (more soluble) and controversies exist concerning to how long HA persists in the body; reports from the orthopedic literature indicate that β-TCP provides better results [9]. However, β-TCP is known to be an excellent promotor of osteoblastic formation and is readily resorbed by macrophages and osteoclasts. Direct bone matrix anchorage has been shown with collagen fibers deposited in the micropores [10]. Resorption of β-TCP by giant cells has been recently reported in a rabbit model with cells having or not a ruffled border (a characteristic of osteoclasts) [10].

Non-steroidal anti-inflammatory drugs (NSAIDs) have analgesic, antipyretic and anti-inflammatory properties. NSAIDs reduce fever, pain and they prevent inflammation. The ectopic new bone formation in the soft tissues around the hip joint can also be prevented by using NSAIDs [11]. A 14-day post-surgery treatment with ibuprofen has been shown to reduce the occurrence of chronic pain and disability with a low risk of adverse effects. NSAID treatments are simple and cheap to administer. However, a number of studies have reported adverse effects of NSAIDs which can delay the consolidation of fractures or the incorporation of the biomaterials because they interfere with the bone remodeling, in particular by their action on the production of prostaglandins [12–16]. In addition, these drugs have been found to impair tendon healing in preclinical studies [17].

The aim of this work was to study the effect of a NSAID, ketoprofen, on the osteoconduction induced by a graft of β-TCP in a model of bone defect in the rabbit. Fluorescent microbeads of a biocompatible polymer (poly 2(hydroxyethyl methacrylate)-pHEMA) were injected at the time of euthanasia to appreciate the macrophagic function in the grafted area.

2. Materials and methods

All chemical reagents were obtained from Sigma–Aldrich Chemical (Illkirsh, France). Commercial 2-hydroxyethyl methacrylate (HEMA) contains impurities, due to the fabrication process.

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The polymerization inhibitor 4-methoxyphenol (added by the manufacturer before shipping, at a concentration of 350 ppm) also needs to be removed. HEMA was purified and distilled under reduced pressure. Benzoyl peroxide (BPO) was recrystallized in methanol twice before use to remove impurities.

2.1. Preparation of pHEMA microbeads

Microbeads of pHEMA labeled with Nile red were prepared according to our previously reported protocol with minor modifications [18,19]. Briefly, microbeads were prepared by an emulsion precipitation method by using the monomer HEMA (2-hydroxyethyl methacrylate). All reagents were obtained from Sigma–Aldrich (Saint-Quentin Fallavier, France). Benzoyl peroxide (2% w/v), a polymerization initiator, was dissolved in the monomer under magnetic stirring. The Nile red (2 mg), a fluorescent dye, was added. The diluent solution was made of a toluene/butanol mixture (40/60 v/v) in which a steric stabilizer, ethyl cellulose (3%, v/v), was dissolved. The diluent was heated at 40 °C in a polymerization reactor; thereafter the monomer was added drop by drop in the reactor. Homogenization was done with an incorporated agitator. The mixture was maintained under nitrogen atmosphere for 15 min and temperature was increased to 75 °C. After 2 h, the EGDMA (ethylene glycol dimethacrylate) (3% v/v), a cross-linking agent, was added. Thereafter, the reaction was left to complete for 6 h to obtain microbeads. After this period, microbeads were washed with Histo-Clear II® (a mixture of aliphatic hydrocarbon and distilled essential oils – food grade, reduced citrus odor, National Diagnostics, Atlanta, Georgia, USA) to eliminate monomer residues and to preserve fluorescence. They were centrifuged three times and washed in Histo-Clear II®, before dispersion and drying for 24 h. At the end, a powder containing millions of dried microbeads was obtained.

2.2. Animals and surgical procedure

Eighteen New Zealand rabbits (approximate weight 3.5 kg) were used in this study. They were acclimated for 8 days to the local vivarium conditions and received synthetic food and water ad libitum. Animals were randomly divided into two groups (Fig. 1):

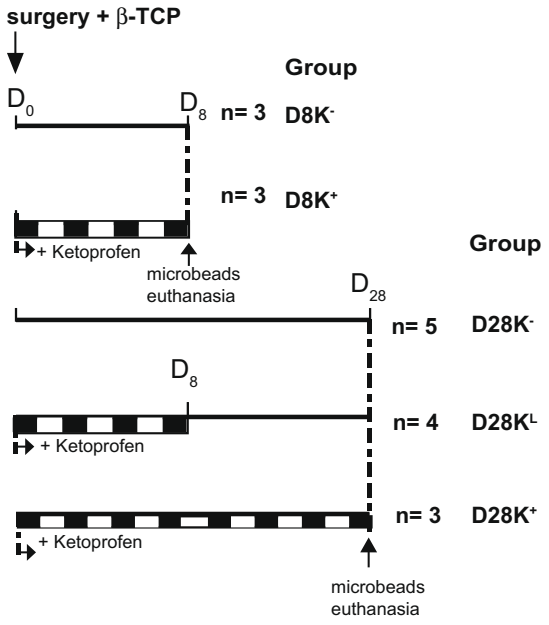

Fig. 1. Flow chart of the present study. Periods of ketoprofen administration appear with a checkerboard pattern.

- 6 animals were operated at D_0 and sacrificed 8 days after surgery to study the early remodeling phase around β-TCP granules. They constituted the D8 group; among them, 3 received ketoprofen (Ketofen® 1%, Merial S.A.S., Lyon, France) (daily dose: 2 mg kg^{-1} subcutaneously) and constituted the D8K$^+$ group, the remaining 3 rabbits did not received the NSAID (D8K$^-$ group).
- 12 rabbits were operated at D_0 and sacrificed 28 days after surgery to study the late remodeling phase around β-TCP granules. They constituted the D28 group; among them, 3 received ketoprofen all along the study (daily dose: 2 mg kg^{-1} subcutaneously) and constituted the D28K$^+$ group; 4 received ketoprofen for a limited period of 1 week post-surgery (D28K^L); the remaining 5 rabbits did not received the NSAID (D28K$^-$ group).

Bilateral femoral implantations were performed under aseptic conditions and general anesthesia with medetomidine (Domitor®, Pfizer, Paris, France) and ketamin (Imalgène 1000®, MERIAL SAS, Villeurbanne, France). After skin incisions and lateral arthrotomy access via the knee joint, a cylindrical defect (4 mm diameter and 6 mm long) was created at the distal femoral end and then filled with 1000–2000 μm β-TCP granules (Kasios, Launaguet-France) (Fig. 2). The granules were placed after the cavity had been flushed with sterile saline to remove debris. Incision was closed by different layers with resorbable sutures. Implantation control was done to ensure that the β-TCP granules have been implanted in the right position.

The animals were sacrificed 8 and 28 days after implantation. 4 h before euthanasia, animals were injected in the ear marginal vein with a saline solution containing 3×10^8 microbeads per ml. The 4 h period was chosen according to a previous study showing

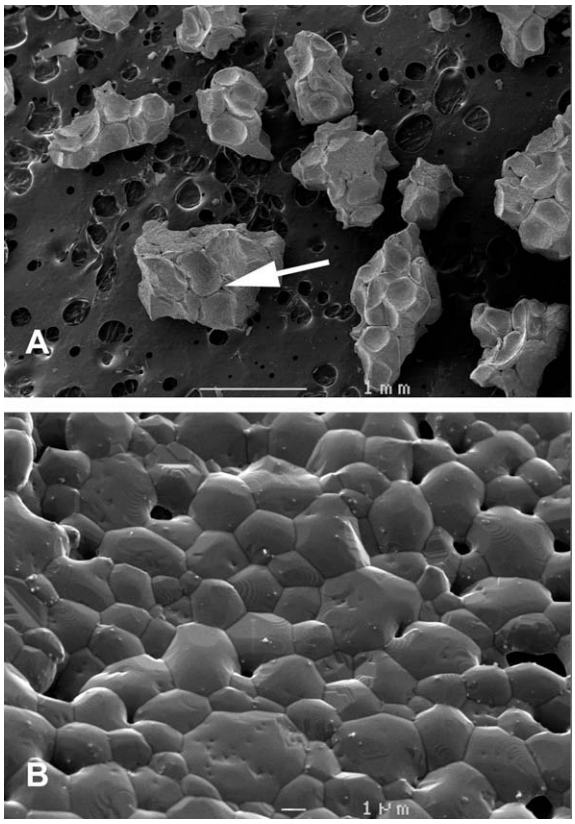

Fig. 2. Granules of β-TCP examined by scanning electron microscopy. (A) Note the general shape of the granules with a grossly polygonal aspect and the small internal voids created by the disappearance of the polyurethane foam during the sintering phase of the biomaterial preparation (arrow). (B) Surface morphology of a β-TCP granule showing the pavement-like structure at higher magnification.

ID	Title	Pages
2297	A non-steroidal anti-inflammatory drug (ketoprofen) does not delay β-TCP bone graft healing	8