USE OF CALCIUM SULFATE AS A BIOMATERIAL IN THE TREATMENT OF BONE FRACTURES IN RABBITS—PRELIMINARY STUDIES

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Abstract

The paper presents preliminary results of the implantation of calcium sulfate type Hartform HF1 developed at the Institute of Glass and Ceramics in Warsaw. The study was conducted on 10 New Zealand White rabbits, in which after tibial osteotomy the biomaterial was implanted directly into the fracture site. The animals were divided into two groups based on the method of fracture stabilisation: intramedullary pinning using Kirschner wires in one group and acrylic external fixator in the other. After 12 weeks, bone union was observed in all animals as estimated by clinical and radiological findings. Histological tests revealed resorption of the biomaterial into the bone fracture area. It was concluded that type Hartform 1 calcium sulfate can be used as a biomaterial in veterinary orthopedics. Due to the lack of observable side effects during implantation and resorption, the material can be considered as a valuable ingredient in composite biomaterials containing calcium sulfate.

Key words: animal model, bone fracture, calcium sulfate, bone union.

Material and Methods

Hartform HF1 gypsum, prepared for implantation at the Department of Bioceramic of the Institute of Glass and Ceramics in Warsaw, was used in the study. The material was a type α gypsum, produced from natural gypsum mineral by means of the autoclave method. This type of gypsum is characterised by a high chemical purity, low liquid-to-powder ratio, and high mechanical durability. Compared to the previously used type β, the structure of type α gypsum offers better mechanical properties, such as compressive strength and tensile strength. Lower porosity and longer time needed to bond with water result in the lowering of the resorption rate of the type α of CS, which makes it more compatible with the natural rate of the bone consolidation (1, 14, 16).

The biocompatibility of Hartform HF1 gypsum was assessed on the basis of a cytotoxicity assay performed by placing CaS implants in a direct contact with mice fibroblasts, as well as based on tissue reaction following implantation into the osseous tissue in rabbits. These examinations were performed at the Department of Experimental Surgery and Biomaterials Research with cooperation with Radiology Department at the Academic Teaching Hospital in Wroclaw.
The study design was approved by the Local Ethics Committee for Animal Care. Tests were conducted on 10 New Zealand White rabbits, male, weighing between 3.5 and 4 kg, aged 9 months. The animals were anaesthetised with a mixture of xylazine (5 mg/kg, intramuscularly) and ketamine (30 mg/kg, intramuscularly), and subsequently by continuous infusion of the mixture of ketamine (30 mg/kg) in 5% glucose (40 mL/kg/h).

Under aseptic conditions the shaft of the tibia was exposed through a medial approach with a minimum damage of soft tissues. After exposing the periosteum in the middle of the tibia, a hole of 3 mm in diameter was drilled. At the lower edge of the defect middle-diaphyseal osteotomy was created with an oscillating saw. The fractures were immobilised with either: two 1.2 mm diameter Kirschner wires (Mikromed) inserted into the medullar cavity in five animals (group 1) or with external fixator type II assembled from 1.2 mm diameter Kirschner wires (Mikromed) and an acrylic frame (Duracryl Spofa) in remaining five animals (group 2). The bone defects and fracture gap were filled with calcium sulfate Hartform HF1. The muscle and fascia were separately sutured with Dexon 3-0 and skin was closed with Ticron 3-0.

After the procedure, x-ray examinations were performed with the Mobilax device (10 mA, 45 kV) using Kodak mammography film. Butorphanol (0.2 mg/kg) for analgesia and trimethoprim/sulfadiazine (30 mg/kg) to prevent infection were given subcutaneously for the first three postoperative days. After 4 weeks from the surgery, radiographs were repeated following premedication with a mixture of ketamine (30 mg/kg), intramuscularly and xylazine (5 mg/kg), intramuscularly. After 12 weeks, the animals were euthanised by intravenous injection of sodium pentobarbital (150 mg/kg). Then, radiological examinations of bone structure were performed and specimens were collected for histological evaluation. During the collection of material, macroscopic examinations of soft tissue appearance in the fracture region were performed. Bone samples for histopathological evaluation were fixed in 10% buffered formalin. The preparations were stained with haematoxylin and eosin and examined under a Nikon-Eclipse microscope.

**Results**

No intraoperative complications occurred either at the moment of implantation or in the entire period of anaesthesia. After 10 d from the surgery, all animals were using the operated limb. Clinical examinations revealed healthy soft tissue structures at the fracture sites without any signs of adverse reaction against the implanted biomaterial. Within the group of animal treated with the use of external fixator, soft tissue swelling around the distal pin was observed in one rabbit, which disappeared after the local treatment and had no influence on the final result of the experiment. Radiological examination, performed 4 weeks after the surgery in all experimental animals, showed normal fracture healing process with the presence of CS in the fracture site. X-ray examinations of animals euthanised 12 weeks after the procedure revealed in all animals well calcified callus in the reconstruction phase and effaced fracture gap. The process of reconstruction of the bone cortical layer and medullar cavity of the tibia was observed. X-ray images revealed no presence of the gypsum material (Figs 3 and 4). Macroscopic examinations revealed bone consolidation in the osteotomised areas. There were no visible macroscopic changes either in the soft tissue surrounding the callus or in the popliteal lymph nodes.

Histological examination revealed the various degrees of resorption of the CS implants. It should be noted that the implanted material was biocompatible. In the regions surrounding bone tissue, the resorption was fusiform (Fig. 1). In the area of the resorption of the implant, demarcation lines were observed between the healthy bone and the implantation area. In the border of this area, an increased number of osteogenic cells was noted (Fig. 2).

No evidence of any adverse histological features characteristic of a foreign body response to the CS was present in either group.

![Fig. 1. Resorbed Hartform HF1 gypsum implant 12 weeks after implantation. Resorption process visible as fusiform. HE, 400x](image)

![Fig. 2. Resorbed Hartform HF1 gypsum implant 12 weeks after implantation. Visible demarcation lines and increased concentration of osteogenic cells. HE, 400x](image)
Calcium sulfate is a biomaterial commonly used in orthopedics, neurosurgery, oncology, periodontology, dentistry, and maxillary surgery (1, 2, 15, 16). The main advantages of this biomaterial include: total resorption in a relatively short period, which roughly corresponds to the duration of the callus formation process, biocompatibility, supply of calcium ions, which promote osteoblastic activity, as well as ease of application and a relatively low cost. Calcium sulfate applied to a fracture, serves as a scaffold for newly forming osseous tissue. It may be used to deliver antibiotics and growth factors (1-3, 8, 15). It has also been used with other materials, such as demineralised bone matrix, autogenous bone, and polymers (1, 2, 15). Moreover, CS is used as an ingredient in composite biomaterials, whose addition can significantly improve their clinical effectiveness (1, 4, 7, 8, 13). Studies of the CS resorption into a bone defect also indicate other beneficial properties, not always displayed by other available biomaterials, such as promotion of angiogenesis (1, 12). The biomaterial facilitates the process of osteogenesis and prevents interposition of the connective tissue into the defect area.

Some authors described side effects involved in the use of biomaterials containing CS, which were observed at the implantation site, such as excessive inflammatory reactions, formation of fistulas and/or wound dehiscence, as well as general complications, such as laryngospasms, tachyarrhythmias, or temporary systemic venous thrombosis (SVT) occurring immediately after implant injection (1, 5, 6, 11). Therefore, there is a need for developing modern biomaterial forms containing CS. Standard animal tests performed before a substance was approved for clinical use involved implantation of the biomaterial into an artificial defect in the osseous tissue, often only in the cancellous bone. It does not always reflect the conditions under which the biomaterial will be used in clinical patients. Therefore, some discrepancies between in vivo results from experimental animals and further clinical trials conducted on humans could occur (10). For such reasons in the described experiment the biomaterial was introduced directly into the fracture site and the healing process was further complicated by the creation of a bone defect. In the study, it was decided to create a model of a comminuted fracture where biomaterial is commonly required to fill the defect. The resorption of the biomaterial was tested under conditions of impaired marrow circulation caused by intramedullary pin, as well as by direct contact between the implanted material and the metal pins in the medullary cavity. In the second group of animals, external fixators were used to recreate the biomechanical conditions typical for elastic stabilisation and eliminate the influence of metal implants in the fraction site.

On the basis of previous reports (1, 7, 9, 13, 15, 16), it was decided to terminate the assessment after 12 weeks from the implantation, a period in which bone consolidation should occur under clinical conditions. After comparing the histological and radiological results, it was concluded that the processes of bone consolidation and resorption of Hartform HF1 gypsum was normal regardless of the stabilisation method applied. On the basis of radiological and histological evaluation, it can be assumed that the analysed...
biomaterial was resorbed after 12 weeks from the implantation, which is consistent with data on CS resorption available in literature (1, 2, 7, 15, 16) and additionally corresponds with the period of normal bone consolidation. The biomaterial was resorbed into the newly formed callus and no adverse reactions during the implantation and resorption were noted.

The study revealed that CS type Hartform HF is a proper biomaterial for the fracture treatment in rabbits, using two different systems of fixation. Because of the lack of side effects during the whole experiment, it was concluded that Hartform HF gypsum may be used as a component of composite biomaterials.

References