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Demineralized bone matrix and platelet-rich plasma do not improve healing of osteochondral defects of the talus: an experimental goat study

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SUMMARY

Objective: The purpose of this study was to evaluate the effectiveness of demineralized bone matrix (DBM) with and without platelet-rich plasma (PRP) in the treatment of osteochondral defects (OCDs) of the talus. We hypothesized that treatment with DBM would result in more bone formation than no treatment in control OCDs, and that PRP would further enhance the regenerative capacity of DBM.
Method: A standardized 6-mm OCD was created in each talus of 16 adult goats. According to a randomization scheme, one OCD of each goat was treated with allogeneic DBM hydrated with normal saline ($n = 8$) or hydrated with autologous PRP ($n = 8$). The contralateral OCD ($n = 16$) served as control. After 24 weeks, the animals were euthanized and the tali excised. Various outcome parameters were analyzed with use of macroscopic evaluation, micro-computed tomography (μCT), histology, histomorphometry, and fluorescence microscopy.
Results: None of the analyses revealed statistically significant differences between the groups for any of the parameters analyzed in any volume of interest. For example, the mean bone volume fraction (BV/TV) of the defect, as measured by μCT, was 0.56 (95% confidence interval [CI], 0.44–0.68) for DBM hydrated with normal saline and 0.52 (95% CI, 0.40–0.65) for DBM hydrated with PRP, compared to 0.53 (95% CI, 0.45–0.61) and 0.54 (95% CI, 0.44–0.64) for the internal controls, respectively ($P > 0.05$).
Conclusion: In contrast to our hypotheses, no beneficial treatment effect of DBM with or without PRP was found for OCDs of the caprine talus.

Introduction

In the treatment of talar osteochondral defects (OCDs), repair of the subchondral bone is an important aim of the procedure[1]. The presence of subchondral bone is essential for survival of chondrocytes[2]. The affected subchondral bone is thought to cause the pain[3]. Restoration of the subchondral bone may improve the weight-bearing capacity of the ankle and prevent further cyst formation.[3]

Urist pioneered the use of demineralized bone matrix (DBM) for bone defects[4,5]. Since his work, there has been increasing experience with DBM in both animals[4,6–8] and humans[9,10]. The sequence of events after implantation of DBM mirrors that of endochondral ossification[4]. Bone morphogenetic proteins (BMP-2, -4, and -7) seem to be responsible for the formation of bone and possibly cartilage that are induced by DBM[11,12]. The BMPs present in DBM attract mesenchymal stem cells through chemotaxis and act as morphogens that may direct the differentiation of these cells into an osteochondrogenic lineage. Different fluids can be used for rehydration of the DBM before application, including normal saline, bone marrow aspirate, antibiotics solution, whole blood, or platelet concentrate.

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Platelet-rich plasma (PRP) is a promising biomaterial that contains concentrated growth factors, including transforming growth factor-β (TGF-β) and platelet-derived growth factor (PDGF)[13,14]. TGF-β in PRP may stimulate chemotaxis and mitogenesis of osteoblast and chondroblast precursors and inhibit osteoclast formation and bone resorption[15,16]. PDGF may promote mitogenesis, angiogenesis, and chondrocyte proliferation[15–17]. Although, in theory, PRP may enhance the biologic activity of DBM, the combination of DBM and PRP has had contradictory results.[15,18,19]

The purpose of the present study was to evaluate the effectiveness of DBM with or without PRP in the treatment of ankle OCDs in goats. We hypothesized that (1) treatment with DBM would repair more bone than control OCDs, and that (2) PRP would further enhance the regenerative capacity of DBM.

Materials and methods

Animals and experimental design

The study was approved by the Animal Care and Use Committee of the University of Amsterdam. A caprine model was used, specifically designed for ankle OCDs[20]. Sixteen adult female Dutch milk goats (Capra Hircus Sana) were included with an approximate age of 4 years. All goats were healthy, according to physical examination and blood tests performed by a veterinarian. The goats were weighed on a digital scale before surgery and at final follow-up. Surgery was performed in a sterile manner on both ankles, with the goat under general anesthesia with endotracheal intubation. A single intramuscular dose with prophylactic antibiotics (Pen & Strep, Fendigo sa/nv, Brussels, Belgium) was injected preoperatively. The ankle joint was exposed through a posteromedial approach. Normal articular surfaces were confirmed by visual inspection. Standard OCDs of 6 mm in diameter and depth were created with specially developed instruments[20]. According to a predefined randomization scheme, one defect of each goat was treated with DBM hydrated either with normal saline (0.9% NaCl solution) ("DBM treatment"; $n = 8$) or with PRP ("DBM + PRP treatment"; $n = 8$), and the other served as a control ("DBM control" or "DBM + PRP control"). In each case, the material was inserted press-fit up to the level of the adjacent cartilage surface. The joint capsule and skin were closed in a standard fashion.[20]

During recovery, the animals were kept outdoors in a large natural environment, without activity restrictions, and with food *ad libitum*. Eating habits, ambulatory activities, and health status were monitored daily.

Since previous studies showed no substantial change in repair of knee OCDs after 24 weeks[21,22], the goats were euthanized 24 weeks after surgery by injecting a lethal intravenous dose of pentobarbital. All analyses were performed by observers blinded to the treatment provided.

DBM

Commercially available cortical DBM (Bonus™ DBM, Biomet BV, the Netherlands) was used. This DBM was obtained from human donors from qualified tissue banks that were registered with the FDA and accredited by the American Association of Tissue Banks. It was granulated, demineralized with organic solvents, freeze-dried (i.e., lyophilized) and processed aseptically. This process resulted in calcium levels of less than 0.1%[12]. It was combined with a collagen-derived carrier (gelatin) from the same donor, packaged in a rehydration syringe, and sterilized by gamma irradiation.

PRP

Autologous PRP was used for rehydration of the DBM. After induction of anesthesia and before surgery, 27 ml venous goat blood was aspirated into a 30-ml syringe that contained 3 ml of anticoagulant citrate dextrose A. PRP was isolated by centrifugation at 3200 rpm for 15 min using the gravitational platelet system II (GPS II, Biomet BV, the Netherlands). This preparation system produces 3 ml of PRP with a reported eightfold increase in platelet concentration and a fourfold to sevenfold increase in growth factor concentration compared with whole blood[23–25]. The concentration of platelets in the PRP of each subject in the present study was measured using an automated hematology analyzer (XE-5000, Sysmex, Japan) after 5 min of resuspension on a rocker, as recommended by Woodell-May *et al.*[24] The median platelet concentration of the PRP was 1511×10^9/l (range, $82–2090 \times 10^9$/l).

Macroscopy

After the goats were euthanized, the tali were excised and digital high-resolution photographs were taken of the talar articular surfaces. Two independent observers macroscopically graded the photographs with use of the validated International Cartilage Repair Society (ICRS) cartilage repair assessment[26,27]. This score ranges from 0 to 12 points and is subdivided into degree of defect repair, integration to border zone, and macroscopic appearance (4 points each), with a score of 12 indicating a completely normal appearance. The scores of the two observers were averaged and outliers with a difference of more than 1 point were scored by consensus.[28]

Micro-computed tomography (μCT)

The anterior part of the talus, at safe distance from the OCD, was sawn off with a water-cooled band saw to reduce the size of the specimen, allowing it to be placed in the μCT scanner, and to optimize penetration of fixative into the specimen. After 1 week in fixative (4% phosphate-buffered formaldehyde), the specimens were submerged in 70% ethanol and temporarily subjected to a vacuum. In the 70% ethanol solution, they were placed in a μCT scanner (μCT 40, Scanco Medical AG, Bassersdorf, Switzerland) and scanned with a resolution of 18 μm. To minimize the noise in the reconstructions, an integration time of 1000 ms was used.

μCT reconstructions were segmented with a threshold level of 467 mg HA/cm^3. Two 3-dimensional cylindrical volumes of interest were defined: one representing the complete OCD (6 mm in diameter and depth), and one representing the central OCD (3 mm in diameter and 5 mm in depth) (Fig. 1). Using morphometric software (Scanco Medical AG, Bassersdorf, Switzerland), the following parameters were analyzed: bone volume fraction (BV/TV, bone volume (BV)/tissue volume (TV)), tissue mineral density (TMD) of BV and of TV, and trabecular number (Tb.N), thickness, and separation. The BV/TV was the primary outcome of the study.

Histology

After fixation, the specimens were dehydrated using ascending grades of alcohol and embedded in methyl-methacrylate. After cold polymerization, the undecalcified specimens were cut into 5-μm sections with a Jung-K microtome (R. Jung, Heidelberg, Germany). Thirty central sections were obtained of each specimen to overcome sampling error. Every third section was stained with Goldner's trichrome method or toluidine blue for light microscopy, or left unstained for fluorescence microscopy. Two observers simultaneously assessed the stained sections and identified the type of healing.