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Assessment of Reamer Irrigator Aspirator System (RIA) filtrate for its osteoinductive potential in a validated animal model

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ABSTRACT

Purpose: Previous studies indicate that Reamer Irrigator Aspirator (RIA) filtrate contains proteins that have the potential to stimulate bone healing. This study aimed to determine the osteoinductive capabilities of RIA filtrate in a validated in vivo model.
Methods: With Institutional Review Board approval, RIA filtrates from 9 patients were collected. The filtrate was processed to remove cells and inorganic particles. A portion of each sample was set aside for protein analysis while the remainder was lyophilized and prepared for implantation. With Animal Care and Use Committee approval, athymic mice (n = 16; 32 hind limbs) were randomly assigned to 1 of 4 groups (n = 8 limbs per group) for percutaneous gastrocnemius muscle injection of demineralized bone matrix (DBM) (10 mg), lyophilized RIA powder (10 mg), RIA liquid (10 mg of lyophilized RIA powder in 100ul phosphate buffered saline (PBS)), or DBM (10 mg) + RIA liquid (10 mg in 100ul PBS). Radiographs were obtained 2, 4, and 8 weeks after injection. At 8 weeks, mice were sacrificed and the entire gastrocnemius muscle from each hind limb was collected and processed for histologic examination. Histological sections and radiographs were assessed for ossification/calcification. Data were compared for statistically significant ($p < 0.05$) differences among groups and strong ($R > 0.7$) correlations between outcome measures.
Results: The protein composition of RIA filtrates was consistent among patients and matched previous data. For all groups, radiographic scores were significantly ($p < 0.014$) higher (more calcification/ossification) at 8 weeks compared to 2 weeks. Radiographic scores for the DBM and DBM + RIA liquid groups were significantly higher than RIA liquid and RIA powder at 4 weeks and 8 weeks ($p < 0.019$ and $p < 0.049$, respectively). Histologic scores were significantly ($p = 0.004$) higher in the DBM + RIA liquid group compared to the RIA liquid group at 8 weeks. Histologic scores showed strong correlations ($r > 0.77$) to radiographic scores for all groups.
Conclusion: RIA filtrate liquid and powder were osteoinductive in vivo with new bone formation being most abundant using a combination of DBM and RIA filtrate in this validated animal model. RIA filtrate has potential for clinical use in augmenting bone healing treatments.

Introduction

The Reamer Irrigator Aspirator (RIA) (Depuy/Synthes, West Chester, PA, USA) is a surgical instrumentation system that uses negative pressure to circulate fluid through the intramedullary canal during reaming of long bones. The device was designed to decrease intramedullary pressure and temperature compared to traditional methods of reaming in order to reduce the incidence of fat embolization and thermal necrosis [1–3]. The RIA system is also commonly used to collect autologous bone graft as an alternative to iliac crest bone graft. A filter attached to the RIA outflow tubing collects and isolates bone particles from the remainder of the circulated fluid so that it can be used for autologous implantation to treat segmental bone defects or nonunions. The remaining filtrate, or "waste water," collected in the suction canister is typically discarded, as it currently has no approved clinical use.

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Previous studies have examined the properties of RIA filtrate [4–8], which contains viable osteogenic cells that can be cultured in media to synthesize a protein profile that is similar to that of cells from iliac crest bone graft [8]. In addition, RIA filtrate alone (without undergoing cell culture) contains proteins with osteoinductive properties, including osteocalcin, osteopontin, osteoprotegerin, and adiponectin [8]. Prior protein analyses has indicated that the RIA filtrate is unlikely to be pro-inflammatory because it contains anti-inflammatory soluble receptors sEGFR, sgp130, sIL-6R, and sTNFRII [8]. As such, acellular RIA filtrate may represent another by-product of the RIA system with the potential to be developed as a novel therapeutic for augmentation of bone healing. This study was designed to test whether RIA filtrate is osteoinductive. We hypothesized that derivatives of RIA filtrate collected from patients during approved RIA procedures would be osteoinductive in an athymic mouse muscle implantation model. We further hypothesized that RIA filtrate would increase the ability of commercially-available DBM to promote osteogenesis in vivo.

Materials and methods

Sample collection

After obtaining Institutional Review Board (IRB) approval and informed consent, 9 patients (6 females, 3 males, mean age 43.3 years, range 25–74 years) were enrolled for RIA filtrate collection. All patients underwent treatment involving use of autologous bone graft for treatment of nonunions or fractures with segmental bone loss. Bone graft was harvested from the femurs using the RIA system according to the manufacturer's technique guide. A single pass through the medullary canal was made for each bone graft harvest. A reamer blade with a diameter approximating the diameter of the isthmus was selected, and between 1 and 3 l of normal saline was used to collect the bone graft. After passing through the filter connected to the outflow tubing, the RIA filtrate was collected in a suction canister and processed for protein analysis and implantation.

RIA filtrate processing

Four 50 ml aliquots were taken from each patient's total volume of filtrate and centrifuged at 2500 RPM for 5 min. Five 2 ml aliquots were taken from each of the centrifuged aliquots and centrifuged again at 10,000 RPM for 5 min. The supernatant from each tube was combined and transferred to a new 50 ml tube, and one 1 ml aliquot was stored at −80 °C for biomarker and protein content analyses; the remainder was kept at −80 °C overnight. After freezing, the RIA filtrate in the 50 ml tube was lyophilized to create a powder (RIA powder) and then stored as individual patient samples at −80 °C until used for in vivo study.

Analysis of RIA filtrate proteins

RIA filtrate samples were analyzed using three commercially available Luminex multiplex assays (EMD Millipore, Billerica, MA, USA) according to the manufacturer's protocols. These assays measure osteoinductive, pro-inflammatory and anti-inflammatory proteins as follows:

- Bone metabolism proteins: adrenocorticotropic hormone (ACTH), dickkopf-related protein 1 (DKK1), insulin, leptin, osteoprotegerin (OPG), osteocalcin (OC), osteopontin (OPN), sclerostin (SOST), parathyroid hormone (PTH), fibroblast growth factor (FGF)-23;
- Cytokines: FGF-2, vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), granulocyte-colony stimulating factor (GCSF), granulocyte-macrophage colony-stimulating factor (GMCSF), growth-regulated alpha protein (GRO-α), interleukin (IL)-6, IL-1β, IL-1α, platelet-derived growth factor (PDGF)-AA, PDGF-BB, tumor necrosis factor (TNF)-β, TNF- α;
- Soluble cytokine receptors: sCD30, sEGFR, sgp30, sIL-1RI, sIL-1RII, sIL-2Ra, sIL-4R, sIL-6R, sReceptor for advanced glycation end-products (RAGE), sTNFRI, sTNFRII, sVEGFR1, sVEGFR2, sVEGFR3.

In vivo testing

With institutional animal care and use committee (IACUC) approval, athymic mice (n = 16) were anesthetized using 5% isoflurane in oxygen and prepared for aseptic implantation of test substances into both gastrocnemius muscles. Mice were randomly assigned to one of four treatment groups (n = 8 per group):

1. lyophilized RIA powder (10 mg) alone;
2. RIA liquid (10 mg lyophilized powder in 100ul PBS) alone;
3. DBM (10 mg) (DBX paste, Depuy/Synthes, West Chester, PA, USA) alone;
4. a combination DBM (10 mg) and RIA liquid (10 mg lyophilized powder in 100ul PBS).

For implantation, each test substance was percutaneously injected into the center of each gastrocnemius muscle using a 14 gauge needle based on palpation (Fig. 1). Mice were recovered from anesthesia, provided analgesics (buprenorphine 0.05–0.1 SQ mg/kg q12 h, carprofen 4–5 mg/kg SQ prn) and individually housed in conditions appropriate to their immunocompromised status, including microisolater cages, autoclaved bedding and nests, irradiated diet, and barrier facility.

Mice were anesthetized again at 2, 4, and 8 weeks after implantation and radiographs were obtained in mediolateral views of each hindlimb using a standardized high-detail technique for digital radiography (Progeny Dental VetVision DC, Lincolnshire, IL).

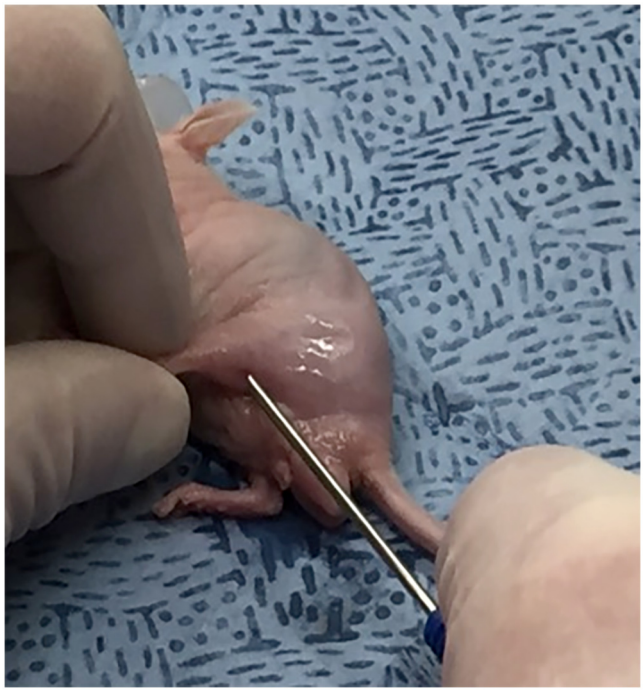

Fig. 1. Percutaneous injection into the center of the gastrocnemius muscle of a study mouse.

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