Assessment of RIA Filtrate Osteoinductive Potential in an Ectopic In Vivo Model

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Purpose: The reamer-irrigator-aspirator (RIA) is a device popularly utilized for harvest of autologous bone graft. RIA filtrate has been noted to contain multiple biologically active mediators that could be important for stimulating bone growth. We aimed to assess the osteoinductive potential of RIA filtrate in a validated in vivo model.

Methods: After IRB and animal care and use committee approvals, the liquid filtrate from patients undergoing medullary reaming of the femur utilizing the RIA system was collected. The RIA filtrate was then processed for protein analysis and implantation into the muscles of mice. Filtrate samples were assayed for the presence of multiple factors known to be associated with bone growth, including but not limited to adrenocorticotrophic hormone (ACTH), fibroblast growth factor (FGF), interleukin (IL)-1, IL-6, parathyroid hormone (PTH), osteocalcin (OC), osteoprotegrin (OPG), and vascular endothelial growth factor (VEGF). Athymic mice (n =16; 32 hindlimbs) were randomly assigned to 1 of 4 test groups (n = 8 limbs per group). Mice were anesthetized for percutaneous intramuscular implantation into the center of each gastrocnemius muscle with either demineralized bone matrix (DBM) (10 mg), powder lyophilized from RIA liquid (10 mg), RIA liquid (10 mg of filtrate in 100 μL phosphat- buffered saline (PBS), or DBM (10 mg) + RIA liquid (10 mg in 100 μL PBS). Radiographs of both hindlimbs were obtained at 2, 4, and 8 weeks after implantation. Mice were euthanized at 8 weeks and the entire gastrocnemius muscle from each hindlimb was collected and processed for histologic examination. Histological samples and radiographs were blindly rated according to a semiquantitative scheme.

Results: RIA filtrates were obtained from 9 subjects (6 females, 3 males; mean age 43.3 years; range, 25-74 years). The protein composition and concentrations of samples was consistent among patients and contained proteins important for bone production. All mice were successfully implanted and survived for the intended duration of study. No complications were noted. For all groups, radiographic scores were significantly ($P < 0.014$) higher (more ossification) at 8 weeks compared to 2 weeks. Radiographic scores were not significantly different among groups at 2 weeks. However, DBM and DBM + RIA 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 group compared to the RIA liquid group at 8 weeks; otherwise, histologic scores were not significantly different between groups. Histologic scores showed strong correlations ($r > 0.77$) to radiographic scores for all groups.

Conclusion: RIA filtrate safely induced new bone formation in the muscles of athymic mice. New bone formation was greatest in muscles injected with a combination of DBM and RIA proteins. RIA filtrate alone did not induce new bone formation to the same degree

as DBM in this model. However, both RIA liquid and RIA powder were able to induce new bone formation in muscle, and this significantly increased in amount and maturity over the 8-week study period. RIA filtrate and lyophilized RIA powder appear to be osteoinductive. We recommend validation for clinical use through further testing of the osteoinductive potential of RIA filtrate in a critical-sized defect animal model.

See pages 49 - 106 for financial disclosure information.