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CORR Insights

CORR Insights®: D-amino Acid Inhibits Biofilm but not New Bone Formation in an Ovine Model

Jessica Amber Jennings PhD

Where Are We Now?

Complex musculoskeletal injuries are complicated by infections in as many 30% of traumatized patients [2], depending on the type of injury and the timing of the interventions. Biomaterials often are necessary components to restore function and promote healing in injuries where muscle, nerve, and bone damage present challenges to healing. While biomaterials serve critical functions in repairing defects, they also increase the risk of infection. This is in part due to the promotion of biofilm formation on the surface of implants, and in particular, the presence of persister cells (dormant cells that survive exposure to antimicrobials) within biofilm. Biofilm microorganisms and persister cells have altered metabolic function that make them resistant to traditional antibiotic therapy and require antimicrobial concentrations several orders of magnitude higher for complete eradication than those necessary for killing planktonic counterparts [9].

Basic science studies of biofilm and persister-cell biology have led to the discovery of a number of molecules that specifically target these phenotypes. D-amino acids (D-AAs), cis 2-decenoic acid, and farnesol are among these recently discovered biofilm inhibitors that have been shown to prevent biofilm formation, disperse existing biofilm, and revert persister cells to a more active and antimicrobial-susceptible state [4, 7, 8]. While in vitro results using D-AAs are encouraging, clinical use of these biofilm inhibitors remains investigational. Dose considerations and compatibility with bone and soft tissue must be established for safety. Since systemic delivery of D-AAs and other biofilm inhibitors may be ineffective, local delivery carriers for D-AAs are an advantageous strategy for clinical use.

Where Do We Need To Go?

Recent studies of antiinfective strategies for implanted materials have highlighted the need for inclusion of biofilm-targeting therapy in addition to antibiotics [1, 5]. In the study by Harmata and colleagues, the specific inhibition and dispersal of both methicillin-susceptible and methicillin resistant *Staphylococcus aureus* strains

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J. A. Jennings PhD (✉)
Department of Biomedical Engineering, University of Memphis, 330 Engineering Technology Building, Memphis, TN 38152, USA
e-mail: jjnnings@memphis.edu

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were confirmed at particular D-AA concentrations. As a preliminary in vitro measure of compatibility, dose-dependent effects of D-AAs on osteoblasts and osteoclasts were measured over the course of several weeks. Harmata and colleagues did observe effects on cell number and cell function comparable to other similar studies [10], including decreases in alkaline phosphatase-positive, osteocalcin, collagen, osteoblast colonies, and tartrate-resistant acid phosphatase staining. However, the effects occurred at concentrations higher than those required for antibiofilm activity and further occurred at a repeated full-dosing schedule. Sustained delivery of these higher concentrations during the course of several weeks would be difficult to achieve with many local delivery systems. The results of this study highlight the need to control release or administration of biofilm inhibitors in order to maintain concentrations within a target range over which antibiofilm activity is present and cytotoxic effects are minimized.

Because of its ability to provide sustained release of active molecules compared to antibiotic-loaded autograft or allograft, a low viscosity bone graft substitute under investigation (not FDA approved) was chosen as a delivery system for D-AAs in this study. While specific release profile was not characterized in this study, previous work using a similar bone void filler has demonstrated D-AA release as well as efficacy in inhibiting bacterial adherence [11]. Elution of amino acids in this previous study followed a burst pattern release of 25% to 60% of incorporated D-AAs, with extended elution of lower amounts during 30 days. While it is difficult to accurately predict in vivo release kinetics and tissue concentration of released D-AAs, this difference between the in vitro study dosing and the likely release profile of D-AAs from the low viscosity bone graft implants in the sheep study may explain why negative effects on osteoblasts and osteoclasts were not observed during the healing process in the sheep model in this investigation. Validation of efficacy against biofilm in infected animal models is necessary to confirm that this formulation would indeed prevent infection with minimal effects on bone healing.

How Do We Get There?

We need expanded preclinical investigations to translate this technology and help achieve its clinical potential, including evaluation of different time points as well as confirmation of efficacy using models of infected musculoskeletal trauma. Following this, Phase I clinical trials may demonstrate compatibility and efficacy in maintaining contamination-free healing of bone using low viscosity bone grafts.

Increasing evidence also suggests that antibiotics may be a necessary addition to ensure elimination of contamination [3, 4, 6]. While reduction in biofilm biomass was observed using D-AAs alone, complete eradication was not observed even at the highest concentrations studied. Even with effective dispersal or inhibition of biofilm, any remaining cells could rebound and seed further sites of biofilm formation. Combinations of D-AAs and particular antibiotics have been shown to increase efficacy of both against biofilm [8]. Studies identifying appropriate antibiotics for inclusion with D-AAs may lead to more effective prevention strategies, but will require additional validation of biocompatibility.

Although release and compatibility of D-AAs were demonstrated in the present study for an investigational bone graft substitute, other delivery systems must be developed to expand the clinical applicability of this antibiofilm technology. Demonstration of activity and release of D-AAs from approved and more commonly used local delivery biomaterials, such as polymethylmethacrylate, calcium sulfate, or polymeric beads and hydrogels may provide a quicker route for translation of antibiofilm therapeutics.

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Further, development of other types of drug-delivery systems may expand the applications beyond those for which a bone graft substitute would be appropriate. Coatings on implants, injectable degradable polymers, or sponge-based delivery systems might provide benefits of D-AAs for multiple applications. As growing awareness of the role of biofilm and persister cells in infection is increasing clinical demand for biofilm-specific prevention and treatment strategies, approaches such as the D-AA modified bone graft substitute demonstrate great promise in reducing the rate of infectious complications.

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