•Superoxide Dismutase Mimetic Disrupts Bacterial Biofilms in an Infected Fracture Model

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Background/Purpose: Implant-associated infections affect more than 50,000 orthopaedic cases a year. *Staphylococcus aureus* is one of the most common pathogens and its ability to persist locally and resist antibiotics is related to its ability to form and maintain biofilm structures. Fixation devices and total joint components provide a surface for bacterial adherence and foster bacterial growth within a 3-dimensional extracellular structure that is phenotypically different from its planktonic (single cell) counterpart. Biofilm associated infections become chronic and difficult to diagnose, leading to fracture nonunions and premature failures of total joints. Reports that bacteria actively modulate their redox environment in a biofilm by downregulating superoxide dismutase (SOD) suggested a novel treatment for biofilms. We postulated that a potent SOD mimetic would interfere with either the establishment or maintenance of the biofilm structure and might improve clinical outcomes. We tested the compound (MnTE-2-PyP) in an in vitro model and a murine infected fracture model with and without antibiotics.

Methods: *In Vitro:* A biofilm-forming subtype of *S. aureus* (ATCC 29213) was used. Biofilm assessment was done using crystal violet assay for extracellular polymeric structure (EPS); *S. aureus* was diluted and plated on sterile 96-well PVC plates. Cultures were grown over 24 hours, treated with drug (30 μM) or PBS (phosphate-buffered saline) at baseline or after 12 hours of growth. Absorbance was read at OD595 using a microplate reader. *Animal Model*: Procedures were approved by the Institutional Animal Care and Use Committee at National Jewish Health. Male C57BL6 mice (20-25 grams) were used. A midshaft femur fracture was created through a lateral incision and then treated with intramedullary fixation using an 8-mm section of 23-gauge needle. 10^3 bacteria in 5 μl volume were placed at the fracture site and the soft-tissue envelope was restored with 6-0 Vicryl and skin glue. There were four treatment groups and five mice per group: (1) no drug treatment, (2) SOD mimetic alone (MnTE-2-PyP), (3) cephalexin 250 mg/mL administered in drinking water, and (4) MnTE-2-PyP and cephalexin. The animals were allowed unrestricted activity for 2 weeks. Femurs were harvested at the end of 2 weeks. Bone was dissected free of surrounding muscle, weighed, homogenized, sonicated, then plated for quantitative cultures.

Results: Mn TE-2-PyP disrupted established biofilms (after 12 hours of growth) in vitro at both 15 and 30 μM concentrations. Neither dose prevented the formation of the initial biofilm structure. In the infected fracture model, mice regained full weight bearing within 24 hours when fixation was adequate. Treatment with cephalexin alone reduced the bacterial counts at 2 weeks by 75% compared to no drug treatment, but there were residual bacteria cultured in all of the animals. In the MnTE-2-PyP with cephalexin group, bacterial cultures were zero in all animals at 2 weeks ($P < 0.0001$)

Conclusions: An SOD mimetic drug in combination with standard antibiotic treatment is more effective than antibiotics alone for treating a biofilm associated bone and implant

See pages 99 - 147 for financial disclosure information.

infection. In vitro work suggests that the drug interferes with maintenance of the biofilm EPS structure, which may allow improved antibiotic penetrance as well as improved immune cell activity.

- The FDA has not cleared this drug and/or medical device for the use described in this presentation (i.e., the drug or medical device is being discussed for an "off label" use). For full information, refer to page 600.