INTRODUCTION: Infection is a devastating orthopaedic complication when associated with fractures stabilized with internal fixation. Infection impairs fracture healing. In addition, bacteria adhere to the surfaces of a metallic fixation device and synthesize a glycocalyx, insulating them from host and antibiotic assault. Effective treatment of the infection often requires removal of the fixation device, but this would be counterinductive until the fracture has attained some degree of stability. An intervention that would accelerate fracture healing in the presence of an infection would represent a significant advance in the successful treatment of infected fractures. The goal of this study was to examine the ability of osteogenic protein-1 (OP-1) to induce the formation of de novo bone in the presence of bacterial infection in an intramuscular model in the rat.

MATERIALS AND METHODS: This work was approved by our institution’s Animal Care and Use Committee. A 1 cm-long pocket was surgically created in the musculus longissimus thoracis on both sides of the spine in each of 120 male Sprague-Dawley rats (total of 240 pockets). The two pockets in the 72 animals in one group did not receive a metal implant, while the pockets in the 48 animals in the second group did (2 mm-length of 2 mm-diameter stainless steel tubing). Half of the animals in both groups received 5 x 10^5 colony-forming units of Staphylococcus aureus in 0.05 ml of sterile saline, while the pockets in the other half did not. The pockets with/w/o bacteria in each animal received either no OP-1, or 10 or 25 µg of recombinant OP-1 dissolved in 0.1 ml of sterile water. The OP-1 solution and/or suspension of bacteria were mixed with 60 mg of lyophilized bovine collagen carrier, and then this moistened collagen was packed into the muscle pocket with/w/o the metal implant. Six animals with each combination of no bacteria/bacteria, OP-1 dose, and metal were euthanized at 1, 2, 3, or 4 weeks postoperatively. The tissue samples were harvested and fixed in 10% formalin. High-resolution radiographs of the tissue samples were obtained using a Faxitron system (Hewlett-Packard, McMinnville, OR). The areas of newly formed bone in the tissue samples were quantified using a BioQuant Workstation (R&M Biometrics, Nashville, TN). The tissue samples were demineralized for 24 hours, and the calcium content of the tissue in the pockets was quantified by testing the demineralized solution with a flame atomic absorption spectrophotometer (AAS) (Varian Instruments, Walnut Creek, CA). The tissue was then processed for histology.

RESULTS: A factorial analysis revealed that the presence/absence of infection, OP-1 dose, and outcome time points significantly affected the calcium content of the tissue in the pockets (p<0.0001) (Figure 1), and the presence/absence of metal did not (p=0.533). The calcium content and radiographic area of new bone formation, in pockets both with and without metal, were significantly greater without infection than with infection, significantly greater with 25 µg of OP-1 than with 10 µg or no OP-1, and significantly greater at 4 weeks than at 1 and 2 weeks (all p<0.05). Both calcium content and area of new bone in the infected pockets were significantly greater with 10 and 25 µg of OP-1, compared to infected or uninfected pockets without OP-1 (p<0.05, Figures 1, 2); OP-1 maintained its osteoinductive abilities in the presence of infection. The action of OP-1 was dose-dependent for the given infection; the calcium content with 25 µg was significantly greater than with 10 µg (p<0.05). Both calcium content and area of new bone formation were significantly greater in pockets with OP-1 without infection at 4 weeks, compared to pockets with OP-1 with infection (p<0.05). Histology showed significant new bone formation in the presence of infection. (Figure 2).

DISCUSSION: OP-1 plays an important role in bone formation by promoting osteoprogenitor cell proliferation and differentiation to chondroblastic and osteoblastic phenotypes. OP-1 maintains its osteoinductive abilities in the presence of infection under the conditions in this study, compared with infected pockets without OP-1, as evidenced by significantly greater amounts of calcium ions in the demineralized solution and radiographic area of new bone formation. This study also showed that OP-1 induced significantly more new bone in the uninfected muscle tissue, compared with the infected tissue, and that the action of OP-1 was dose dependent for the same initial bacterial inoculum. This suggests that the action of the OP-1 is impacted by the infection process to some degree, and that a higher dose of OP-1 may be needed for a greater bone formation response. The factorial analysis revealed that there was no significant effect on the calcium content between the groups of animals with and without metal, suggesting that bone induction by OP-1 is not affected by the metal, with or without infection. The evidence of persistent infection in the tissue in the infection groups was confirmed by positive qualitative bacteriology cultures taken at the time of tissue harvest, and by histological signs of chronic inflammation, including cellular reaction with a preponderance of mononuclear cells, and proliferation of fibroblasts and new vessels. Detection of the level of calcium ions in the demineralization solution by atomic absorption spectrophotometry reflects the amount of bone formation in the tissue.

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Figure 1. Calcium content of tissue in infected pockets with metal was significantly higher at each time point with OP-1, than without OP-1 (p<0.05, n=6 in each group)

Figure 2. Histology shows OP-1 induced new bone formation (B in Figure) at 4 weeks in infected group, while there was no bone found without OP-1, other than the remnants of collagen carrier (C) and inflammatory reaction (I). (H.E., 100 X)