Early Inhibitory Effects of Zoledronic Acid in Tooth Extraction Sockets in Dogs Are Negated by Recombinant Human Bone Morphogenetic Protein

David A. Gerard, MS, PhD, Eric R. Carlson, DMD, MD,† Jack E. Gotcher, DMD, PhD,‡ and David O. Pickett, DMD, MS§*

Purpose: This study was conducted with 2 purposes. The first was to determine the effect of a single dose of zoledronic acid (ZA) on the healing of a tooth extraction socket in dogs. The second was to determine if placement of recombinant human bone morphogenetic protein-2 (rhBMP-2)/absorbable collagen sponge (ACS) - INFUSE, (Medtronic, Memphis, TN) into these extraction sockets would inhibit the inhibition on bone healing and remodeling by ZA.

Materials and Methods: Nine adult female beagle dogs (2 to 3 yr old) were placed into 3 groups of 3 dogs each. Group I received 15 mL of sterile saline intravenously; group II received 2.5 mg of ZA intravenously; and group III received 5 mg of ZA intravenously. Forty-five days after treatment, all dogs underwent extraction of noncontiguous right and left mandibular first molars and second premolars. In group I, the right mandibular extraction sockets had nothing placed in them, whereas the left mandibular sockets had only ACS placed in them. In groups II and III, the right mandibular sockets had rhBMP-2/ACS placed in them, whereas the left mandibular sockets had only ACS placed. All extraction sockets were surgically closed. Tetracycline was given intravenously 5 and 12 days later, and all animals were euthanized 15 days after tooth extraction. The extraction sockets and rib and femur samples were harvested immediately after euthanasia, processed, and studied microscopically.

Results: A single dose of ZA significantly inhibited healing and bone remodeling in the area of the tooth extractions. The combination of rhBMP-2/ACS appeared to over-ride some of the bone remodeling inhibition of the ZA and increased bone fill in the extraction sites, and remodeling activity in the area was noted. The effects of rhBMP-2/ACS were confined to the area of the extraction sockets because bone activity at distant sites was not influenced.

Conclusions: A single dose of ZA administered intravenously inhibits early healing of tooth extraction sockets and bone remodeling in this animal model. The combination of rhBMP-2/ACS significantly increased bone fill and bone remodeling in these areas, negating much of the effect of the ZA.

J Oral Maxillofac Surg 72:61-66, 2014

Received from the Department of Oral and Maxillofacial Surgery, University of Tennessee Medical Center, Knoxville, TN.

*Professor.

†Professor and Chairman.

‡Professor.

§Chief Resident.

This study was funded by Medtronic Corporation, Memphis, Tennessee.

Address correspondence and reprint requests to Dr Carlson: Department of Oral and Maxillofacial Surgery, University of Tennessee Medical Center, Suite 335, Knoxville, TN 37920; e-mail: ecarlson@mc.utmck.edu

Received May 8 2013

Accepted June 10 2013

0278-2391/13/00797-0$36.00/0

http://dx.doi.org/10.1016/j.joms.2013.06.192

Zoledronic acid (ZA) is the most potent of the nitrogen-containing bisphosphonate medications.[1] It is reported to be 20 times more potent than alendronate.[2] ZA binds to apatite crystals in bone[3] and inhibits remodeling by inducing apoptosis of osteoclasts.[4] It persists for long periods on bone surfaces and is estimated to have a half-life longer than 10 years.[5] It is the only known bisphosphonate that is effective in decreasing or preventing lytic bone lesions, as seen in metastatic breast cancer and multiple myeloma,[6-9] and blastic bone lesions associated with metastatic prostate cancer.[10,11] ZA is approved by the Food and Drug Administration (FDA) for the treatment of osteoporosis[12] and has shown antitumor effects in some patients with breast[13] and prostate[14] cancer. Although it has proved to be quite effective in treating these conditions, its use has been associated with the development of bisphosphonate-related osteonecrosis of the jaws (BRONJ) in patients who have had invasive dental procedures.[15-17]

Bone morphogenetic protein (BMP) was first described by Urist and Strates[18] as a substance extracted from bovine bone that had the capability to induce bone formation in rat muscle pouches. Since that time, more than 20 BMPs have been discovered. Six of these, including BMP-2, are in the transforming growth factor-β superfamily.[19] A recombinant human form of BMP-2 (rhBMP-2) loaded onto an absorbable collagen sponge (ACS)[20] is currently available (INFUSE, Medtronic, Memphis, TN). INFUSE has been approved by the FDA for use in spinal fusion surgery,[21] ulnar open fractures,[22] and around endosseous implants[23] to stimulate bone formation and enhance bone remodeling. BMP-2 appears to stimulate osteoblast formation and activity during bone healing[24] and increases bone remodeling.[25]

A single intravenous dose of ZA is effective in inhibiting bone activity and persists on bone surfaces for years.[5] The present study examined the effect of a single dose of ZA on early tooth extraction socket healing. This study also examined whether placement of rhBMP-2/ACS into the tooth sockets could negate the effects of ZA on early healing and bone remodeling.

Materials and Methods

All procedures used in this study were approved by the University of Tennessee animal care and use committee. Animals were housed in environmentally controlled rooms at a University of Tennessee–accredited Association for Assessment and Accreditation of Laboratory Animal Care facility. The animals were acclimated for at least 2 weeks before treatment. All treatments, including surgery and anesthesia, were closely monitored by institutional animal care and use committee veterinary staff. Animals were maintained on soft diets postoperatively and closely observed for any adverse reactions after surgery.

Nine adult female beagle dogs were used in this study (Marshall BioResources, North Rose, NY). Dogs weighed 25 to 30 lbs and were placed in 3 groups of 3 animals each. Group I, the control group, received 15 mL of sterile injectable saline through the saphenous vein over a 15-minute period. Group II received ZA 2.5 mg (North Drug Store, Vancouver, BC, Canada) dissolved in sterile saline, and group III received ZA 5 mg dissolved in sterile saline in the same manner as group I.

Forty-five days after treatment, each dog was placed under general anesthesia and underwent extraction of noncontiguous right and left mandibular second premolars and first molars. Group I animals had nothing placed in the right mandibular extraction sockets. A 0.5- $\times$ 1-inch section of ACS was placed in the left premolar sockets and a 1- $\times$ 2-inch section of ACS was placed in the left molar sockets. In groups II and III, the right mandibular extraction sockets had the ACS saturated with rhBMP-2 0.07 mg (INFUSE) for the premolar sockets and the ACS saturated with rhBMP-2 0.28 mg for the molar sockets. All tooth extraction sockets were primarily closed.

Animals were euthanized 60 days after the initial treatment. Ten and 3 days before euthanasia, the animals received intravenous tetracycline 10 mg/kg body weight dissolved in sterile saline. Immediately after euthanasia, a portion of the right and left mandible, including both extraction sockets, was harvested and placed in Carson fixative (Fisher Scientific, Kalamazoo, MI). In addition, a central 2-cm portion of the right fifth rib and of the right femur was harvested and placed in fixative.

Samples were fixed for 48 hours and then dehydrated over a 2-week period in an ethanol series. The undecalcified samples were embedded in Spurr embedded medium (EMS, Washington, PA). Two 100-μm- thick longitudinal sections were cut through the center of each tooth extraction socket and the rib and femur samples using a Leitz 1600 bone saw (Ernst Leitz Wetzlar GmbH, Wetzlar, Germany) equipped with a diamond wafering blade. These sections were ground and polished to 40-μm thickness. Sections were examined with an epifluorescence microscope (Carl Zeiss, Jena, Germany) equipped with digital display IMAGE software (National Institutes of Health, Bethesda, MD) to measure bone turnover rates and bone apposition rates. Then, sections were stained with alizarin red and toluidine blue and examined with bright-field microscopy to calculate bone volumes using IMAGE software. These values were compared for the rhBMP-2 versus non–rhBMP-2 sides in groups II and III and with the control group using analysis of variance (n = 6; 2 extraction sites for each group from 3 animals).