Introduction
The natural healing process requires significant augmentation in the treatment of large bone defects resulting from non-unions or surgical removal of bone tumors. Innovative attempts to enhance bone healing usually involve augmentation of one or more of three basic elements that contribute to the natural bone healing response: the supply of osteogenic cells, an osteoconductive substratum and endogenous macromolecular factors involved in osteoinduction, angiogenesis, chemotaxis and cell adhesion. The purpose of this study was to determine the lowest doses of recombinant human bone morphogenetic protein-2 (BMP2), combined with osteoconductive biomaterials, that will heal critical-size diaphyseal defects in a rat model.

Methods
Experimental models of bone healing used in this study consist of segmental defects created in rat fibula by osteotomy and immediately repaired with tubular specimens of demineralized bone matrix (DBM). The DBM specimen, prepared in advance from femur or tibia of an allogeneic animal, is slightly longer than the defect and is fitted over the cut ends of the fibula as a bioactive scaffold. The early sequential stages of normal human bone healing that result in the initial osseous union with woven and lamellar bone are recapitulated in two of the models we developed in previous studies (1,2). In 2mm and 4mm-long defects in the fibula repaired with 5mm and 8mm-long DBM specimens respectively, the DBM scaffold is converted to a neocortex consisting of woven and lamellar bone in approximately 7 to 8 weeks (1,2). Removal of osteoinductive activity of the scaffold by various means results in acute models of impaired bone healing (2). Two experiments were performed in the present study. In the first experiment, a 4mm-long segmental defect was repaired with an 8mm-long DBM scaffold which had been treated to remove osteoinductive activity by extraction with guanidine HCl and mild trypsin digestion (gtDBM). Thirty-two adult male Sprague-Dawley rats (350-400 g b.w.) were divided into 5 to 7 adult male Sprague-Dawley rats (350-375 g b.w.). Three groups were treated with 20 µl of microcrystalline hydroxyapatite (MHA), MHA + 100ng BMP2 and MHA + 500ng BMP2 respectively during surgery and euthanized 7 weeks later. Two untreated control groups were euthanized at 7 and 11 weeks respectively during surgery. The fibulae were tested in three-point bending to determine rigidity. The fibulae were tested in three-point bending to determine rigidity. Subsequently, the mineral content of the repair site was determined. In the second experiment, a 6mm-long defect was repaired with an unmodified 8mm-long DBM scaffold in five groups of 5 to 7 adult male Sprague-Dawley rats (350-375 g b.w.). Three groups were treated with 20 µl of microcrystalline hydroxyapatite (MHA), MHA + 100ng BMP2 and MHA + 500ng BMP2 respectively during surgery and euthanized 7 weeks later. Two untreated control groups were euthanized at 7 and 11 weeks respectively. Bone repair was evaluated as in the first experiment. A standard bioassay (3) to determine the osteoinductive activity of BMP2, as measured by ectopic osteogenesis in abdominal wall muscles of the rat, was performed in separate groups of rats. All experimental protocols had been approved by the R&D and Animal Studies Committee of the Omaha VA Medical Center.

Results
In the first experiment (Figure 1) chitosan alone increased the rigidity by 92% (p=0.034). Treatment with chitosan and 100ng BMP2 increased the rigidity by 74% above that of chitosan alone (p=0.003). The corresponding result for 500ng BMP2 was an 83% increase in rigidity (p=0.0002). The difference between the two doses of BMP2 was not statistically significant. In the second experiment (Figure 2) the 100ng dose combined with MHA was ineffective. But MHA + 500ng BMP2 increased the rigidity significantly over the MHA-treated control at 7 weeks (p<7x10^-5), untreated 7-week control (p=5x10^-7) and untreated 11-week control (p=0.02). The bioassay demonstrated that 100ng of BMP2 had significant (p<0.028) osteoinductive activity.

Discussion
The models used in both experiments involved critical size defects since the diameter of the fibula at mid-diaphysis is 1.5 to 2.0mm. In the first experiment, the lower dose of BMP2 was adequate to achieve complete healing apparently because of significant contributions to the bone repair process by the osteoconductive properties of chitosan and the gtDBM scaffold. In the second experiment, the volume of the defect was approximately 50% larger, but the DBM scaffold was unmodified and hence contained BMP and other growth factors. This may have resulted in slightly higher rigidity of untreated bone in this experiment at 7 weeks compared to that in the first experiment. The failure of MHA + 100ng dose of BMP2 to stimulate bone repair in this model may have been due to slow resorption or incorporation of MHA. However, when MHA was combined with 500ng BMP2, the higher dose of the growth factor apparently overcame this adverse effect. The rigidity achieved by this treatment is comparable to that in our normal healing model consisting of a 4mm defect repaired with an 8mm unmodified DBM scaffold. The main finding of this study is that a relatively low dose (100 to 500ng) of BMP2 combined with appropriate osteoconductive biomaterials may be adequate for complete healing of large bone defects.