THE EFFECT OF VASCULAR ENDOTHELIAL GROWTH FACTOR ON THE HEALING OF CRITICAL SIZE LONG BONE DEFECTS IN RODENTS

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Introduction: Vascular endothelial growth factor (VEGF) is one of the most potent angiogenic growth factors that has been identified to date. As such, it is being intensely studied for use in a wide range of medical conditions that involve an impaired or damaged blood supply. In the orthopaedic arena, its expression has been identified during fracture healing in humans as well as rodents. Additionally, studies have shown that both osteoclasts and osteoblasts are responsive to variations in VEGF levels, and rodents lacking certain isoforms suffer from impaired endochondral bone formation. It has also been shown to act synergistically with members of the BMP family. However, despite its obvious importance in skeletal growth and repair, no in vivo data exists regarding its potential therapeutic role in the healing of large bone defects. The objective of this study was to examine the effects of VEGF on the healing of critical size defects in the rat femur.

Materials and Methods: Surgical Procedure: A 5 mm critical size defect was created in the right femur of 20 male retired breeder Sprague-Dawley rats. The femur was stabilized using a custom-fabricated polymer bone plate, which was held in place with 4 stainless steel screws and cerclage wire. Implant Materials: In group A (n=8) the defect was filled with a collagen sponge (Helistat, Integra Lifesciences) soaked with carrier. Group B animals (n=8) had the defect filled with a collagen sponge that was soaked with 0.5 µg VEGF in the same volume of carrier. Group C animals (n=4) had the defect filled with a collagen sponge and 2.0 µg VEGF. Recombinant human VEGF 165 (R&D Systems) was used in all cases. Serial Radiographs: All animals were examined using microradiography every two weeks. Radiographs were taken using a Hewlett-Packard Faxitron and Kodak MinR-2000 mammography film. Histology: An equal number of animals from each group were sacrificed at 4 and 8 weeks post-surgery. After the femurs were collected and cleaned of extraneous soft tissue, the samples were fixed, dehydrated, embedded in polymethylmethacrylate and processed for undecalcified histology. Sagittal ground sections were prepared and stained with Stevenel’s Blue and Van Gieson’s picro-fuschin. Histomorphometry: The area of new bone formation was measured on two slides per specimen using the Bioquant image analysis program (R&M Biometrics, Nashville, TN). Seven specific regions were measured on each slide in order to track bone formation from key locations. Data Analysis: Results were analyzed using an ANOVA at each time point (StatView, SAS Institute, Cary, NC).

Results: Radiographically, there were no demonstrative differences between the control group and either of the VEGF doses. In all cases there was very little if any new bone visible on the microradiographs, and none of the defects had approached healing. The histomorphometry data showed that there was no difference in the total amount of new bone formed between the three groups at either of the time points, as shown Figure 1. In fact, at 4 weeks, the 0.5 µg and 2.0 µg groups produced 52% and 51% less bone than the control group respectively. Most of the decrease in new bone formation at this time point appeared to be arising from a reduced amount of endosteal bone in the VEGF groups. On average however, both of the VEGF groups actually had more new bone in the defect area at 4 weeks. In three of the 4-week animals treated with 0.5 µg VEGF, mineral was seen forming on the sponge in the center of the defect as shown in Figure 2. In the other two groups, the sponge was not present in any of the specimens and had apparently already resorbed. At the 8 week time point there was a relatively larger increase in the amount of new bone produced in the 0.5 µg group as compared to the 2.0 µg group. At this time the 0.5 µg only had 13% less bone than the controls while the 2.0 µg group still had roughly half (48%) as much new bone as the controls. The most interesting finding however, was the presence of apparently de novo bone “islands” in three of the four animals which received the 0.5 µg VEGF dose at this time point, an example of which is shown in Figure 3. These isolated bone formations were very well formed and contained vascular channels filled with red blood cells and mature marrow elements.

Discussion: These results indicate that, at the dosage levels studied, VEGF administered alone does not have significant osteogenic effects in this model. In fact, the amount of new bone formed was reduced at both time points studied with both of the VEGF doses. Additionally, the data suggest that the 2.0 µg dose may have been more inhibitory than the 0.5 µg dose as there was a tendency for greater improvement with the 0.5 µg dose at 8 weeks. However, despite these findings, the animals which received the 0.5 µg dose of VEGF frequently had unique patterns of bone formation in the center of the defect. At 4 weeks post-op, the sponge appeared to be acting as a scaffold for bone growth that may have resulted in the islands of bone seen in several of the 4-week animals. This may indicate that VEGF does have some limited osteogenic potential in vivo, however, additional studies with lower doses of VEGF may be necessary in order to confirm this.

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