Introduction  The rate of lengthening has a profound impact on bone formation during distraction osteogenesis. At optimal rates of distraction, palisades of well-vascularized woven bone fill the distraction gap. However, rapid distraction leads to the formation of granulation tissue, fibrocartilage, and cysts, resulting in nonunion. Previous research has demonstrated that rapid rates of distraction decrease the expression of cytokines necessary for angiogenesis and osteogenesis. Some suggest this is due to damage of the periosteum, while others believe it may be related to rapid lengthening relative to the age and size of the patient. In this study, we explored whether a rapid rate of distraction leads to a decrease in the angiogenic and osteogenic growth factors; basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), and vascular marker, platelet endothelial cellular adhesion marker (PECAM-1/CD 31) in a rat limb lengthening model.

Methods  Following IACUC approval, periosteal-sparing mid-diaphyseal femoral lengthenings were performed in two groups of male Sprague-Dawley rats (460-515 grams, age < 6 months). The animals in the first group (n=30) were lengthened at a rate of 0.5 mm/day and the animals in the second group (n=30) were lengthened at a rate of 1.5 mm/day. In both groups, the lengthenings were initiated after a seven day latency period, the lengthenings were performed twice daily, and the overall lengthenings were 7.0 mm. Animals from both groups were sacrificed on post-operative days 8, 10, 12, 14, and 21 (n=6/group/timepoint). At sacrifice, the lengthened femurs were explanted, fixed in zinc formalin, demineralized with EDTA, and embedded in paraffin. Serial 5 µm mid-sagittal sections were cut from each bone. Several sections from each bone were stained with hematoxylin and eosin for routine morphological analysis; the remainder were stained with antibodies against bFGF (sc-79, Santa Cruz Biotechnology), VEGF (sc-507, Santa Cruz Biotechnology), PDGF (sc-128, Santa Cruz Biotechnology), and PECAM-1/CD 31 (sc-1506, Santa Cruz Biotechnology). For the immunohistochemistry, the slides were deparaffinized in xylene and hydrated with a graded series of alcohols. Antigen unmasking was performed using 10 mM sodium citrate buffer at 95 °C. Peroxidase activity was quenched in methanol and hydrogen peroxide before incubating the slides in 1.5% rabbit blocking serum. Slides were then incubated with the primary antibody overnight at 4 °C. The following day, slides were incubated with secondary antibody, followed by avidin-biotinylated horseradish peroxidase. Slides were incubated with peroxidase substrate, then counterstained with hematoxylin and dehydrated with graded alcohols and xylene before coverslipping. Positive and negative (no primary antibody) controls were run concurrently.

The slides were evaluated with a Nikon Eclipse E800 microscope (Nikon Instruments, Inc., Melville, NY) and Spot 3.4 digital imaging system (Digital Instruments, Inc., Sterling Heights, MI). Growth factor expression in the central fibrous zone, primary mineralization front, and peripheral (periosteal) new bone was graded as strong (dark brown, ≥50% cellular staining), moderate (light to dark brown, 25-50% cellular staining), weak (light brown, ≤25% cellular staining), and absent.

Results  The bones from fifty-two (0.5 mm/day, n=26; 1.5 mm/day, n=26) animals were available at the end of the experiment; four animals from each group were lost due to infection or technical problems. Histology  On day eight, granulation tissue appeared in the distraction gap at both rates of distraction. By day twelve there was formation of new bone in the distraction gap and central fibrous zone in the slow distraction specimens (Fig. 1A). In the fast distraction specimens, however, the distraction gap was typically filled with a large, central cyst surrounded by granulation tissue (Fig. 1B). On day twenty-one, longitudinally aligned new bone filled the majority of the distraction gap in the slowly distracted animals, whereas the cysts persisted in the fast distraction specimens and there was little new bone formation.

Discussion  In this study we found cyst formation and decreased growth factor expression with rapid distraction. Our findings suggest that nonunion associated with rapid lengthening may be due, in part, to disruption of the newly forming blood supply, as well as decreased expression of angiogenic and osteogenic growth factors.

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