Delayed union of femoral fractures in older rats:decreased gene expression

ABSTRACT

(1) All genes studied were up-regulated by fracture in both age groups. Thus, the failure of the older rats to heal promptly was not due to the lack of expression of any of the studied genes. (2) The return of the mRNA gene expression to baseline values in the older rats prior to healing may contribute to their delayed union. (3) No genes were overly up-regulated in the older rats. The slower healing response of the older rats did not stimulate a negative-feedback increase in the mRNA expression of stimulatory cytokines.

INTRODUCTION

Background While the radiographic and histologic progression of fracture healing is well understood, there are less data on the cytokines that regulate and control the healing process at the cellular level. The bone morphogenetic proteins (BMP) have been found to be expressed in the fracture callus by immunostaining of the protein as well as by measurement of mRNA and in situ hybridization. The receptors for BMP are also up-regulated during fracture repair. Morone et al. observed increased mRNA expression of both matrix genes and BMP genes during the healing of spinal fusions in rabbits. There was a sequential expression of these genes in that BMP-6 peaked first, followed by BMP-4, then BMP-2, and last by a second peak of BMP-6 expression. The data for BMP expression during fracture repair, combined with the efficacy of the BMPs in stimulating bone induction and fracture healing, have led to the concept that the BMPs are key molecules in the initiation of this healing process. Derangement of the expression of BMPs and other skeletally active cytokines may be important in understanding the failure of healing in delayed unions. It is not clear why, in some fractures, the healing process seems to come to a halt and does not progress further without surgical intervention. We have found that younger rats heal mid-shaft femoral fractures faster than do older rats. In these earlier studies, one-year-old rats failed to achieve normal biomechanical strength within 24 weeks after mid-shaft femoral fracture. In contrast, young rats achieved normal biomechanical strength by four weeks after fracture. In this model, as the rats get older, it takes progressively longer for them to begin a periosteal reaction after fracture, and it takes longer to achieve bridging callus. This slowing of fracture healing with age has been reported in humans as well as in rats. In humans, the time to union increased with age for humeral fractures. The reason for the slowing of fracture healing in older animals is not fully understood. For some time it has been known that there are changes in the periosteal cell layer with age. Following fracture there is an increase in the rate of mitosis in the periosteum near the fracture site. This rate slows with age: Fewer cells enter mitosis, and more time is required for the cells to undergo mitosis. This may reflect a reduction in the number of osteogenic stem cells available for skeletal repair in older individuals. Alternatively, changes in the expression of the cytokines controlling fracture healing would also affect the rate of healing. Recent work has shown that the genes responsible for embryonic induction of skeletal tissue are also involved in fracture healing. The BMPs are prominently expressed during embryonic tissue induction (reviewed by). BMP-2 and BMP-4 have been reported in the fracture callus of rats and mice. This led to the hypothesis that the delayed fracture healing in older individuals may be related to abnormalities in the BMP-signaling pathway. To test this

hypothesis, bone fracture was induced in young and older rats, and gene expression was measured in the fracture callus at various times after fracture induction. Expression of osteocalcin, type I collagen, and type II collagen were measured as markers of fracture callus formation. BMP-2, BMP-4 and the type IA BMP receptor were measured as potential regulators of the healing process.

CONCLUSION

Conclusions In summary, mid-diaphyseal fractures of the femur in rats were followed by increased mRNA expression for osteocalcin, type I collagen α1, type II collagen, BMP-2, BMP-4, and the type IA BMP receptor. In the younger rats, there was elevated gene expression until fracture healing occurred. In contrast, in the older rats, fracture healing was slower, so that cytokine gene expression returned to baseline prior to radiographic or biomechanical healing of the fractures. This decreased expression of bone-inductive genes prior to an adequate biological response suggests the absence of negative feedback regulation over fracture healing.