Chapter 9

Summary

Bone is one of the few tissues capable of complete regeneration. The mechanisms behind this phenomenon are of great interest not only in understanding the processes of bone repair and bone metabolism, but also in gaining insight into the regeneration of non-skeletal tissues. Growth factors are now generally accepted to play a crucial role in regulating bone formation and bone resorption. Nevertheless, the cascade of growth factors dictating bone regeneration still needs to be elucidated. Distraction osteogenesis, in which bone formation is induced under gradual distraction of two bone surfaces, can be used both as an orthopaedic treatment option and as a model to investigate the basics of bone regeneration experimentally.

In **Chapter 1** the aims of this thesis are presented. First aim of this thesis was to evaluate the clinical results of treatment of antebrachial growth deformities with a distraction osteogenesis procedure and to identify prognostic factors to predict the functional outcome in dogs with these growth deformities. Second goal was to investigate the role of local and systemic growth factors during distraction-induced bone regeneration. Third aim was to stimulate bone healing in a critical-sized bone defect model.

In **Chapter 2** a review of literature is provided covering relevant knowledge of bone histology and histiogenesis, growth plate injuries, healing of bone fractures, distraction osteogenesis, circular external skeletal fixation, hormonal regulation of bone formation, skeletal growth factors, and bone markers.

The results in **Chapter 3** demonstrate that incongruity of the elbow joint and osteoarthritis of the elbow and antebrachiocarpal joint are major complicating factors in treating antebrachial growth deformities in dogs. Treatment with a circular external skeletal fixation system was effective in correcting angular and rotational growth deformities. Nevertheless, it was not possible to completely restore all antebrachial length deficits in these patients with the distraction procedure. Limb alignment and function improved in all dogs. Elbow and antebrachiocarpal osteoarthritis progressed despite surgical correction of the growth deformity. Initial elbow osteoarthritis, initial function, radial length deficit, and ulnar length deficit were identified as prognostic factors in dogs with antebrachial growth deformities. These factors should be addressed to predict the functional outcome of treatment with a distraction osteogenesis procedure.

In **Chapter 4** the expression is reported for growth hormone (GH), growth hormone receptor (GHR), insulin-like growth factor-I (IGF-I), insulin-like growth factor-II (IGF-II), and bone morphogenetic protein-2 (BMP-2) in distraction-induced bone regenerate. Expression of these factors was assessed during the consolidation phase, comparing distraction osteogenesis with new bone formation induced by an osteotomy. In addition, plasma GH profiles and plasma concentrations were determined for IGF-I, IGF-II, and insulin-like growth factor

binding protein- 4 (IGFBP-4) and IGFBP-6. Expression of GHR in the distraction-induced bone regenerate was significantly higher than in osteotomy-induced new bone. Expression of GHR, IGF-I, and BMP-2 in the distraction-induced bone regenerate and in the newly formed bone induced by osteotomy had increased compared with the expression of these factors in mature bone. Circulating levels of GH, IGF-I, IGF-II, IGFBP-4, and IGFBP-6 did not change during the distraction osteogenesis procedure. We conclude that up-regulation of GHR expression may enhance sensitivity to endogenous systemic GH and thus promote consolidation of the bone regenerate after distraction osteogenesis.

In **Chapter 5** the efficacy of the bone markers osteocalcin (OC) and carboxyterminal cross-linked telopeptide of type I collagen (ICTP) is assessed in monitoring bone formation during distraction osteogenesis in dogs. Commercially available immunoassay kits for OC and ICTP were used to monitor bone formation and bone resorption, respectively, during a distraction osteogenesis procedure and during bone healing of an osteotomy. The radiographic amount of newly formed bone was determined using densitometric image analysis. Plasma levels of OC and ICTP did not reflect the differences in the amount of newly formed bone. We concluded that the bone markers OC and ICTP are not effective in monitoring bone formation and bone resorption, respectively, in this canine model of distraction osteogenesis.

In **Chapter 6** delayed-image bone scintigraphy is evaluated to quantitatively assess distraction-induced bone formation. Delayed-image bone scintigraphy is a non-invasive method to monitor changes in bone metabolic activity. Bone scintigraphy relies on the uptake of technetium-99m tracer by newly formed bone. Delayed-image bone scintigraphy was conducted during a distraction osteogenesis procedure and compared with healing of an osteotomy. Scintigraphic ratios were calculated and compared with the amount of bone formation as determined with densitometric image analysis. Distraction osteogenesis and bone healing of an osteotomy resulted in increased delayed-image scintigraphy ratios not only in the affected crus, but also in the adjacent femur. Delayed-image bone scintigraphy was not effective at differentiating between the amounts of distraction-induced bone and osteotomy-induced bone. We conclude that delayed-image bone scintigraphy is not adequately sensitive to quantitatively monitor bone formation, but may be useful as an early predictor of bone healing.

Growth hormone plays an important role in bone metabolism. Treating bone defects is a major topic in orthopedic surgery. In **Chapter 7** we hypothesize that local continuous GH administration stimulates bone healing in a canine critical-sized bone defect model. Bone formation in the defects was quantified with densitometric image analysis and histomorphometry. Treatment with GH resulted in healing of bone defects, but without an additional effect of local infusion.

Expression of IGF-I was lower in the bone regenerate of GH treated dogs, whereas IGF-II and GHR expression were not increased. Growth hormone administration increased circulating levels of IGF-I and IGF-II. We conclude that continuous infusion of GH stimulates bone healing in this critical-sized bone defect model. Local application of GH did not additionally enhance bone healing in this model. Increased plasma concentrations of IGF-I and IGF-II most likely induce bone formation.

In **Chapter 8** the results of the presented studies are discussed in the context of their hypotheses postulated in **Chapter 2**.