Chapter 8

General Discussion

Bone is capable of complete regeneration after destruction of its architecture. This ability of bone to repair itself is the basis of distraction osteogenesis in which a bone regenerate is formed under gradual distraction of two bone segments.[30,31] The mechanisms orchestrating bone regeneration are of great interest not only in understanding the process of bone repair, but also in gaining insight into the regeneration of non-skeletal tissues. Distraction osteogenesis can be used as an orthopaedic treatment option, but also as a model to investigate the basics of bone regeneration experimentally.

Antebrachial growth deformities (AGD) are the most common limb malformation in dogs.[32] These growth deformities are characterized by a combination of antebrachial length deficit, angular and rotational malalignment, elbow incongruity (EI), and carpal subluxation. Clinically, AGD results in compromised limb function and altered cosmetic appearance. In these dogs, lameness is the result of a combination of antebrachial length deficit, limb malalignment, and joint pain. The treatment of AGD is aimed at correcting length deficits and limb malalignment, at restoring joint function and at preventing secondary degenerative changes in the joints. The concept of distraction osteogenesis, using a circular external skeletal fixation (CESF) system, proved to be very effective in veterinary orthopaedics.[38,39,44] Our experience with distraction osteogenesis in correcting canine AGD dates back to 1994. The aim of the study presented in **Chapter 3** was to evaluate distraction osteogenesis in correcting AGD and to determine prognostic factors in treating these deformities. At presentation, dogs with AGDs are typically less than 7 months of age, which implies functional growth plates and thus growth potential in the contralateral antebrachium. The growth potential of the contralateral limb should be taken into account during the distraction procedure to compensate for the remaining growth. Realignment of the mechanical axis of joint movement and reduction of EI and carpal subluxation should be achieved as soon as possible. Incongruity of the elbow joint will lead to malformation, which is not amenable to correction. By analogy, carpal subluxation results in malformation of the antebrachiocarpal joint. Established OA is a major factor in the outcome of AGD treatment. Preventing OA is therefore critical in these dogs. Carpal OA after distraction of the antebrachium could be attributed to compression of the antebrachiocarpal joint.[24,51] Careful monitoring of imminent antebrachiocarpal flexor contracture is recommended. In many cases distraction was ended prematurely for this reason.

Summarily, AGDs can be treated successfully with a CESF lengthening procedure despite small remaining length deficits. Treatment limitations are mainly determined by the pre-existing OA and malformation in the elbow and carpal joints. Initial elbow OA and initial limb function are prognostic factors in predicting functional outcome. The cosmetic appearance after treatment is

determined by the magnitude of the initial radial and ulnar length deficits. This study determined the medium-term function after AGD treatment. Progression of elbow and carpal OA may have a negative effect on the long-term functional outcome.

Knowledge about the role of osteotropic growth factors in relation to distraction osteogenesis remains limited. In **Chapter 4** we hypothesized that distraction osteogenesis differs from osteotomy bone healing in the expression of growth factors in the bone regenerate and also differs in the circulating levels of these factors.[18,20,21,37,41,49] In order to gain insight into the regeneration of bone we determined the expression of GH, GHR, IGF-I, IGF-II, and BMP-2 in distraction-induced and osteotomy-induced bone regenerate. In addition, plasma GH profiles and plasma concentrations of IGF-I, IGF-II, IGFBP-4, and IGFBP-6 were determined to assess their potential systemic role during bone formation. The role of GHR in the growth plate has been addressed recently.[12,26] Our study is the first to demonstrate enhanced expression of GHR in distraction-induced bone regenerate. This finding is in agreement with the first part of our hypothesis. Up-regulation of GHR expression in distraction osteogenesis may enhance sensitivity to endogenous systemic GH and thus promote consolidation of the bone regenerate. Our study supports the concept of a direct effect of GH on bone.[29,54] Treatment with GH was effective in stimulating bone formation after distraction osteogenesis, which is consistent with up-regulation of GHR.[7,8,48] Our study was limited by the fact that the expression of GH, GHR, IGF-I, IGF-II, and BMP-2 was determined in the consolidation phase of distraction osteogenesis only. Gene expression should ideally be evaluated continuously to elucidate the role of these factors during active lengthening and during maturation of the bone regenerate. In addition, pursuing other osteotropic and angiogenic factors will be essential to a further understanding of osteogenesis. We reject the second part of our hypothesis in this chapter as changes in the circulating levels of the osteotropic growth factors GH, IGF-I, IGF-II, IGFBP-4, and IGFBP-6 do not seem to play an important role during distraction osteogenesis.

In **chapter 5** we hypothesized that the bone markers OC and ICTP are effective in monitoring bone formation. Commercially available immunoassay kits for OC and ICTP were used to determine bone formation and bone resorption, respectively, during distraction osteogenesis. Ideally, these bone markers should be able to determine early bone formation, to assess progression of bone consolidation, and to predict the outcome of bone healing. Although OC is considered to be an osteoblast-related marker of bone formation, its precise function is unknown.[3,11] In contrast, osteocalcin-deficient mice demonstrated increased bone formation.[14] Matrix metalloproteinases are responsible for type-I collagen breakdown thus releasing ICTP.[25] Age is an important biological factor of

bone marker variation in dogs.[2,3] Clear circadian rhythms were demonstrated for OC and ICTP in dogs.[36,40] Reports concerning bone markers to monitor osteogenesis in dogs are limited to OC, ICTP, and BAP.[17,23,37,47] The hypothesis stated in this chapter was discarded as plasma concentrations of OC and ICTP did not correlate with the amount of bone regenerate induced after distraction osteogenesis. The markers OC and ICTP were not effective in monitoring bone formation in this canine model. The marker BAP was effective in predicting the progression of osteosarcoma in dogs.[17] This marker looks promising as a candidate to assess bone formation and the possibilities of BAP should be explored in more detail.

Delayed-image bone scintigraphy is a non-invasive quantitative method for evaluating changes in bone metabolic activity.[22,33] In contrast to radiography, which reveals the amount of mineralization, delayed-image bone scintigraphy evaluates uptake of technetium-99m tracer by newly formed bone and thus precedes actual accretion of bone.[45,53] Delayed-image bone scintigraphy has been used successfully during distraction osteogenesis to predict the progression of bone formation in the early stages of the lengthening process and to assess the optimal time of bone consolidation in the later stages of bone maturation in human patients.[22,33] In **Chapter 6** we hypothesized that delayed-image bone scintigraphy is effective in quantitatively monitoring bone formation after distraction osteogenesis. In addition, we speculated that distraction osteogenesis, which is known to increase local and regional blood flow, increases bone metabolism in the adjacent long bone.[4] Although blood supply is considered closely related to rate of osteogenesis, blood flow, as indicated by the perfusion index, appears to be an unreliable predictor of new bone formation.[6,22,33] In our study, delayed-image bone scintigraphy was not effective in quantitatively differentiating between distraction-induced bone formation and osteotomy-induced bone formation, thus rejecting the first hypothesis of this chapter. Nevertheless, increasing delayed-image bone scintigraphy ratios were consistent with the radiographic evidence of advancing bone formation.[22] Increased metabolic bone activity in the adjacent femur was demonstrated not only after distraction osteogenesis, but also during osteotomy-induced bone healing. Placement of a CESF on the crus resulted in a similar increase of bone metabolism in the femur as induced after distraction or osteotomy. In view of this, the second hypothesis in this chapter was accepted. Whether enhanced bone metabolic activity was the result of production of angiogenic and osteotropic growth factors is unclear. Although delayed-image bone scintigraphy may be clinically valuable as an early predictor of bone healing, quantification of bone regenerate in individual patients does not appear to be feasible.

Dealing with bone deficits is a major concern in orthopaedic surgery. In **Chapter 7** we hypothesized that continuous GH infusion is effective in stimulating

bone healing in a critical-sized bone defect model. In addition, we speculated that local administration of GH by its direct effect on the GHR is most effective in enhancing bone healing. We expected GH to stimulate the expression of IGF-I, IGF-II, and GHR within the original bone defect and to alter circulating plasma concentrations of IGF-I, IGF-II, IGFBP-4 and IGFBP-6. Our study demonstrates that continuous infusion with GH stimulates bone formation and bone healing in a critical sized bone defect, thus confirming the first part of the hypothesis in this chapter. In contrast to our second hypothesis, local delivery of GH with an infusion pump does not additionally enhance bone healing. Growth hormone treatment did not increase the expression of IGF-I, IGF-II, and GHR in the bone regenerate during the consolidation phase. Plasma concentrations of IGF-I and IGF-II were increased during GH treatment. Although the stimulation of bone healing with GH has been reported previously, our study was the first to show the effectiveness of GH on bone regeneration in a critical-sized bone defect.[7,8,13,48,55]

Expression of GHR was similar in GH-treated dogs and controls and is consistent with the concept of a direct effect of GH on bone regeneration. The fact that local infusion with GH into the defect had no additional effect on bone healing may be attributed to redistribution of GH into the circulation. In our study, IGF-I expression levels were lower during the consolidation phase of the GH-stimulated bone regenerate. In theory, this suggests that IGF-I production in the bone regenerate was not responsible for the progression of bone healing at this stage. Bone accretion progressed despite the fact that IGF-I plasma concentrations returned to preoperative levels after cessation of GH infusion. These findings are consistent with a role of IGF-I during the early stages of callus formation.[10] Whether IGF-I production in the bone regenerate during GH treatment plays an important role in comparison with circulating liver-derived IGF-I remains unclear. In contrast to IGF-I, IGF-II plasma concentrations remained elevated even after cessation of GH infusion. As IGF-II expression did not differ between the GH treated defects and the controls, sustained IGF-II production in skeletal or even non-skeletal tissues outside the defects could be partly responsible for enhanced bone regeneration. Although systemic treatment with GH has proved effective, local routes of GH application focusing on the direct effect of GH merit further research.

Recently, several reports have demonstrated the crucial role of GH and GHR in non-skeletal tissues. The stimulation of liver tissue regeneration with GH in particular has received substantial attention.[34,43] Transgenic rats with GH deficiency demonstrated a decreased reparative response after administration of an hepatotoxic drug.[52] In transgenic mice with blocked GH action, regeneration of liver tissue was dramatically reduced, whereas mice with blocked IGF-I action demonstrated a normal regenerative potential.[46] Stimulation of liver regeneration

with GH was more effective than treatment with IGF-I and a direct effect of GH was proposed in rats.[5] Proliferation of old-age liver after GH stimulation was mediated through forkhead box m1b.[35] Treatment with GH was effective in stimulating liver regeneration after hepatectomy in human patients with hepatocellular carcinoma.[42] In epidermal tissues GH was used to stimulate regeneration in skin wounds and in burn patients.[16,27] The action of GH treatment in skin tissue was mediated directly with local production of IGF-I in the epidermal tissue contributing to the healing process.[15] Growth hormone and GHR were demonstrated to play an important role in the regeneration of several tissues of the digestive tract, including gastric and colonic mucosa.[50,56] Treatment with GH stimulated healing of gastric ulcers in rats.[9] The role of GH was also convincingly demonstrated in nerve and muscle tissue.[1,28] Treatment with GH resulted in reversal of thymic involution in a human patient.[19]

Summarily, GH plays an important role in modulating bone metabolism. Part of the effect of GH on bone metabolism and bone regeneration is exerted directly without the intervention of IGF-I. This underscores the crucial role of its receptor GHR in bone. There is increasing evidence to support the role of GH and GHR in both bone tissue regeneration and non-skeletal tissue regeneration. The full potential of GH as a universal stimulating factor of regeneration has yet to be explored.

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