Growth factors in distraction osteogenesis

Immuno-histological pattern of TGF-β1 and IGF-I in human callus induced by distraction osteogenesis

Abstract

Although growth factors have been demonstrated during bone healing, their presence has not yet been confirmed in callus distraction. Therefore, in 3 patients we searched for cytokines during callus distraction. Bone biopsies were immuno-histochemically stained for TGF-β1, IGF-I, TGF-β type II receptor, IGF receptor, and proliferating cell nuclear antigen (PCNA). Histologically we found immature woven bone in the middle of the callus zone and increasing calcification and lamellar bone in the re-modelling zone. Osteoblasts and fibroblast-like cells in the middle zone, and osteoblasts in all zones stained for TGF-β and its receptor. The number of positive staining cells related to proliferous activity as assessed both by PCNA, and by bone density in radiographs. IGF-I could be detected everywhere. In conclusion, growth factors are present in bone formation and in areas of re-modelling during callotasis. Their relation to proliferous activity and radiographic density supports their involvement in osteogenesis.

Résumé

La présence de facteurs de croissance a été montrée dans la consolidation osseuse mais pas encore établie dans les cals par distraction. Nous avons étudié les cytokines durant la distraction chez 3 patients. Des examens immuno-histo-chimiques ont été faits à partir de biopsie pour étudier les facteurs TGF-β, IGF-I, récepteurs TGF-β II, récepteurs IGF et l’antigène nucléaire de prolifération (PCNA). Histologiquement, nous avons trouvé de l’os immature dans la zone médiane du cal et, dans la zone de remodelage une calcification avec os lamellaire. Les ostéoblastes en général et les cellules fibroblastes-like de la zone médiane ont été positives pour le facteur TGF-β et son récepteur. Il existe une corrélation entre la quantité de cellules positives et l’activité proliférative trouvée par la PCNA et par la densité radiologique. Le facteur IGF-I a été détecté ubiquitairement. En conclusion, les facteurs de croissance sont présents dans l’os en formation et en re-modelage des cals par extension. La corrélation entre l’activité proliférative cellulaire et la densité radiographique, souligne leur rôle dans le processus ostéogénique.

Introduction

The interaction of various growth factors during bone healing and re-modelling has been studied extensively in recent years, mostly in animal models [4–6, 10], but clinical studies are limited. Transforming growth factor beta 1 (TGF-β1), insulin-like growth factors I and II (IGF-I and IGF-II) and platelet-derived growth factor (PDGF) have been recently demonstrated during normal human fracture healing [1–3].

Clinically, rapid bone formation can be produced by distraction osteogenesis [13–15, 24] and there is some evidence of the presence of growth factors which results in new bone [11]. However, the occurrence and distribution of cytokines within the area of bone formation have not previously been established in humans. We therefore investigated the patterns of TGF-β1 and IGF-I in the distraction callus of the femur and tibia in 3 patients.

Materials and methods

During necessary revision surgery, bone specimens were obtained from 3 patients undergoing callus distraction. All the patients gave informed consent for the biopsy, and Institutional Review Board Approval was obtained for this study. The specimens were examined using histological and immuno-histochemical techniques.

Case reports

Case 1

A 22-year-old male had been treated in Croatia for a femoral shaft fracture by open reduction and plating. A deep infection led to os-
tis with death of bone and conventional treatment was unsuccessful. In our institution, resection of the necrotic mid-diaphyseal section of the femur was performed and this was followed by callus distraction and segment transport (1 mm/day) using an AO external fixator [22]. Premature consolidation of the distracted callus after 43 days prevented adequate transport and led to revision surgery. The distal part of the distraction area was osteotomised and transport was resumed after 7 days using a faster rate (1.8 mm/day). When transport and docking had been completed at 94 days, fixation was performed with a condylar plate. Bone specimens were taken from the callus during both slow (1 mm/day) and fast (1.8 mm/day) distraction.

Case 2

A 29-year-old male with a grade III A open tibial fracture was treated primarily by external fixation and then several days later by secondary intramedullary nailing. Deep infection and osteonecrosis occurred, and after admission to our hospital local myoplasties and extensive autogenous bone grafting failed to heal the infected non-union. Segmental resection was performed and this was followed by proximal tibial corticotomy and the start of callotasis. As callus formation was poor, the transportation rate was reduced to 0.6 mm/day. Several further operations were necessary due to the recurrence of local infection. Specimens of the distraction zone were taken during the docking operation at 199 days. Calcification of the distraction callus was slow and osseous consolidation eventually occurred.

Case 3

A 19-year-old female presented with chronic destructive osteitis of the distal femur. Diagnosis was confirmed by radiography, CT, MRI and histology. The distal part of the femur, the knee joint and the proximal end of the tibia were resected. This was followed by proximal tibial and femoral corticotomy, and bifocal callus distraction with the transportation segments being moved toward each other. After the re-appearance of a local swelling, investigation revealed a primary B-cell-lymphoma of the distal femur. Severe local necrosis with super-infection and generalised sepsis occurred, and after admission to our hospital local myoplasties and biocional bone grafting failed to heal the infected non-union. Segmental resection was performed and this was followed by proximal tibial and femoral corticotomy and the start of callotasis. As callus formation was poor, the transportation rate was reduced to 0.6 mm/day. Several further operations were necessary due to the recurrence of local infection. Specimens of the distraction zone were taken during the docking operation at 199 days. Calcification of the distraction callus was slow and osseous consolidation eventually occurred.

Histology and immuno-histochemical preparation

Bone biopsies were placed in 4% buffered paraformaldehyde solution for 48 h. They were then decalcified in 20% EDTA solution. When fixed, the tissue was cut into 2 mm serial sections, and these were paraffinised and stained with hematoxylin for further immunohistochemical examination. Sections for histological examination were stained according to von Kossa and Goldner.

The presence of TGF-β1 and TGF-β type II receptors in the biopsies was detected with TGF-β1 rabbit antibody (Santa Cruz Biotechnology Inc., CA, USA) and a TGF-β type II receptor antibody (Santa Cruz Biotechnology Inc., CA, USA). IGF-I and IGF-I receptors were also detected by the use of rabbit antibodies (Santa Cruz Biotechnology Inc., CA, USA). Cell proliferation was revealed by using primary cell nuclear antigen (PCNA) prepared from mice (Oncogene Science, Uniondale, NY, USA) [8].

TGF-β1, TGF-β receptor type II and PCNA immuno-reactivity were demonstrated by using the avidin-biotin-complex (ABC) method with 3,3’-diaminobenzidine (DAB) serving as chromogen [12]. Sections were incubated overnight at 4°C with TGF-β1 antibody (1:100), TGF-β type II receptor antibody (1:100) or PCNA antibody (1:75) followed by biotinylated swine anti-rabbit IgG (Dako, Fluka, Buchs, Switzerland) or biotinylated rabbit anti-mouse IgG (Dako, A/S, Denmark) for 30 min. Finally, sections were incubated with ABC and 0.05% DAB/0.003% hydrogen peroxide (Fluka, Buchs, Switzerland). Negative control slides were incubated with PBS (phosphate-buffered saline solution).

Staining was classified as non, +, ++ or ++++, depending on the intensity.

Results

Clinically, varying degrees of osteogenesis could be induced in all cases by callus distraction. In Case 1, slow distraction led to premature ossification, and this required a second corticotomy in order to allow segmental transportation to continue. A fast distraction rate produced a moderate amount of callus formation (Fig. 1a). In Case 2, in which the infection recurred, callus formation was poor (Fig. 1b), but osseous consolidation was finally achieved. In Case 3, no problems resulted from callus distraction (Fig. 1c).

Histologically, varying amounts of bone formation were observed. While woven bone and lamellar bone could be identified in different areas of the newly formed bone in Case 1 and Case 3, fibrous tissue with only rare bone trabeculae could be found in Case 2. These findings correlated well with the radiographic appearance of the distraction callus. In the central distraction zone of Case 3, where the “youngest” distraction callus was found, a few irregularly shaped osseous structures with little calcification could be seen (Figs. 2a, 2b). In later distraction callus the bone formed was similar to regular lamellar bone and showed widespread calcification (Figs. 2c, 2d).

PCNA-stained cells could be found in osteoblasts adjacent to the newly formed bone trabecula. Fibrous tissue contained fibroblasts with PCNA positive nuclei (Fig. 3a). The relative quantity of PCNA positive cells showed a good correlation to the radiographic appearance of the callus (Table 1). In Case 2, in which failure of the distraction osteogenesis was suspect at an early stage, only a few cell nuclei stained positively for PCNA. In Case 3, in which specimens from the active middle zone and the border zone could be compared, cells in the middle zone stained much more positively for PCNA than in the zone adjacent to normal bone where the osteogenic activity is lower and the newly formed bone is older (Fig. 3b).

Cells staining positive for TGF-β1 could be found in every region of the distraction callus, mostly in osteo-

Fig. 1a–c Plain radiographs of the distraction area at the time of biopsy. a Case 1: Callotasis of the right femur: note the densities of the distraction callus resulting from 2 different distraction rates. b Case 2: Callotasis of the tibia. Impaired bone formation after 190 days of distraction. c Case 3: Callotasis of the femur. Regular bone formation after 90 days of distraction.

Fig. 2a–d Histology of the distraction callus. a Middle area of the distracted zone. Vivid osteogenesis, immature bone structures with irregular trabeculae and fibrous tissue (Masson-Goldner, bar 50 μm). b Middle area of distraction zone. Early calcification (von Kossa, bar 50 μm). c Re-modelling zone adjacent to corticotomy site, lamellar bone structures, and less fibrous tissue surrounding trabecula (Masson-Goldner, bar 300 μm). d Extended calcification in proximal re-modelling zone (von Kossa, bar 300 μm)