HISTOLOGIC ANALYSIS OF DISTRACTION OSTEOGENESIS IN THE FEMORAL SHAFT OF THE IMMATURE RABBIT

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A study of the distraction callus in the femur of the growing rabbit was performed. Thirty animals separated into 10 groups were used. Distraction between both ends of a femoral osteotomy induced the formation of a connective callus which subsequently ossified. This callus disappeared between the fourth and the eighth week after the lengthening was completed. The osteogenesis model was of a mixed type, intramembranous and endochondral, with a predominance of the former. The bone healing and the initial signs of remodeling in the newly formed bony tissue were seen at the end of the first week of distraction. The original cortex was resorbed during the lengthening and was progressively substituted by a new cortex, whose formation and remodeling was not finished 24 weeks after the completion of the distraction. Experimental conditions could explain some of the findings.

Keywords : distraction osteogenesis ; bone lengthening ; remodeling.
Mots-clés : osteogenèse en distraction ; allongement osseux ; remodelage.

INTRODUCTION

Since Codivilla (5) described the first case of bone lengthening in 1905, considerable efforts to improve the surgical technique and advances in research regarding the histologic sequence of findings inside the distraction callus have been described. The histologic investigations have been focused on three points : a) the study of the osteogenesis model (intramembranous or endochondral) inside the interfragmentary gap (1, 2, 3, 7, 9, 11, 12, 14, 16, 18, 24, 26) ; b) the role of the periosteal and endosteal callus in distraction osteogenesis (7, 16, 17, 20, 27) ; c) the remodeling of the newly formed bone tissue (6, 14, 22, 24). However, previous studies have not reported these three histologic findings during the first two weeks of bone lengthening. The sequence of phenomena regarding the mode of ossification within the distraction callus and the role of the periosteum and bone marrow in distraction osteogenesis have not been clearly identified. The purpose of this article is the histological study of the mineralization process from operation until 29 weeks postoperatively.

MATERIAL AND METHODS

The experimental study was carried out on 30 immature New Zealand white rabbits aged 3 months (2300 to 2600 grams). The skeletal growth of these rabbits is normally completed by 7 months (10). The operation was performed under general anesthesia, using an intramuscular injection of ketamin and midazolam. A longitudinal incision was made over the lateral side of the femur. After the muscles were separated the periosteum was incised and carefully separated. Four self-tapping tapered screws were inserted and connected to an external fixator. A transverse osteotomy was performed in the femoral diaphysis with a power saw. The distraction started 7 days after the operation (waiting period), and was maintained for 28 days at a rate of 1 mm every 24 hours. The consolidation of the distracted area was allowed for 24 weeks, with removal of the external device after 12 weeks. During the experiment, full weight-bearing was

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allowed. The rabbits were separated into 10 groups of 3 animals each (including a control group), and each group was killed at weekly intervals during the elongation period and at the fourth, eighth, twelfth and twenty-fourth week of the consolidation period. The femur was decalcified and slices of 4-micron thickness were obtained. From these cuts, hematoxylin/eosin and Masson trichrome stains were prepared.

RESULTS

Group 1 (control group): This group was composed of rabbits aged 3 months. Lamellar bone with bone formation especially by the periosteum was observed.

Group 2 (waiting period): The area between the bone ends was filled with a reparative fibroblastic tissue ("fibrous callus"). Some newly formed osseous trabeculae were observed under the periosteum (fig. 1).

Group 3 (1 week distraction): The periosteum was hypertrophic and there were some bone trabeculae deposited subperiosteally, mainly by membranous ossification. Cartilage spots in the middle of the interfragmentary space appeared in several sections, close to the fibrous callus. Osteoclasts were already remodeling the newly formed osseous tissue starting in the first week of the elongation period (fig. 2). The original cortex showed several areas with bone formation and other foci with bone spicule resorption. The medullary cavity was occupied by very young fibroblastic tissue (fibrous callus), that contained collagen and reticular fibers longitudinally oriented and surrounded by trabecular formation ("ossification front").

Group 4 (2 weeks distraction): The osteogenic reaction was more intense, with an increased number of cartilage foci (fig. 3) and newly deposited bony tissue. The osteolysis had increased in the original cortex, with necrosis appearing in the bone ends where the osteotomy was performed.

Group 5 (3 weeks distraction): Intensive osteoblastic subperiosteal activity was seen, showing the outline of the final cortex ("neocortex"), which was formed by mainly intramembranous and only limited endochondral osteogenesis. There were

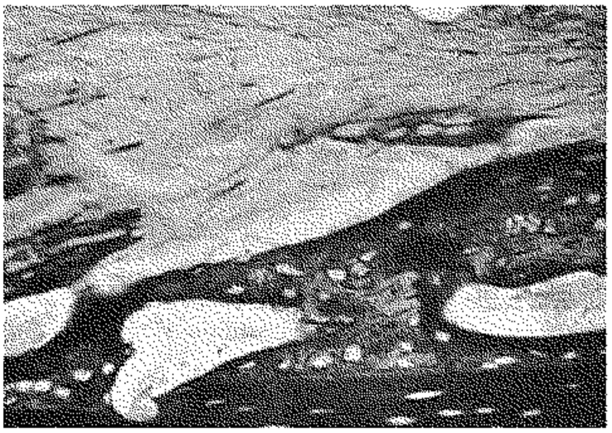

Fig. 1. — Bone trabeculae formed under the periosteum, 1 week after the osteotomy (trichrome, 10 × 20).

Fig. 2. — Newly formed bony trabeculae which are remodeling. Osteoclasts and osteoblasts can be observed (trichrome, 10 × 40).

Fig. 3. — Cartilage foci in the peripheral area of the distraction callus (hematoxylin/eosin, 10 × 6.3).

osteoblasts and osteoclasts in both the neocortex and the primary cortex, with a predominance of osteoblasts in the neocortex and osteoclasts in the original cortex. Bone lamellae were forming at the two borders of the fibrous callus and in the medullary canal, and cartilaginous nodules were differentiating in some areas.

Group 6 (4 weeks distraction): At the end of the distraction phase the periosteum had become thicker ; a higher number of trabeculae and fewer cartilage nodules were present. The original cortex had lost the structure of lamellar bone and showed resorption cavities. The fibrous callus was occupying the central area of the interfragmentary space.

Group 7 (4 weeks consolidation) : The periosteal proliferation showed an involutive process that continued in the following weeks. The neocortex was enlarged and already contained some osteons, with remodeling activity inside. The endosteal and medullary contribution to the new bone deposit had increased and matched the periosteal ossification. Even though its size was much less, the fibrous callus still appeared in the middle of the sections and was replaced by bone healing, deposited almost exclusively by membranous ossification.

Group 8 (8 weeks consolidation): The fibrous callus disappeared, and the columns of intramembranous bone (neocortex) grew towards each other and fused centrally along the lines of the fibrous interzone. The osteolysis continued in the primary cortex, and the bone marrow cavity was now occupied by fatty and hematopoietic marrow cells.

Group 9 (12 weeks consolidation): The neocortex was still maturing and growing, and contained compact cortical bone with a larger amount of osteons.

Group 10 (24 weeks consolidation): Bone deposition in the neocortex was still present, with continued slight remodeling activity inside. The compact cortical bone showed many osteons. Concerning the original cortex, there were only a few residues of bone spicules.

DISCUSSION

The 12-week-old rabbit femur (control group) showed osseous tissue that was maturing. The histologic findings at the end of the latency period were the same as in the initial fracture callus. An outline of structural order in the interfragmentary space was seen after the first week of lengthening, like a hypertrophic fracture callus, with the newly formed collagen bundles aligned in the direction of the distraction, as had been previously demonstrated (11, 14). The electron microscope studies of Ilizarov indicated that the collagen molecules were secreted by cells similar to the type II collagenoblasts, cells typical for embryonic connective tissue (11). Vauhkonen *et al.* (25) studied the composition of the fibrous callus, and they found that it was very similar to the one found in primary bone healing (the healing obtained under stable mechanical conditions). In our study the fibrous callus disappeared between the fourth and the eighth week of the callus consolidation. In other investigations it disappeared 4 weeks after the distraction was stopped, probably because the lengthening period was shorter (16, 17) and the distraction rate was slower (7, 16, 17).

The beginning of trabecular formation in previous studies has been noted between 2 (1, 7) and 3 weeks (18, 20) after surgery, both in histologic and radiologic explorations. The first deposit of bony trabeculae in our rabbits was seen subperiosteally 7 days after the osteotomy. These differences probably result from the fact that we took our first biopsy 1 week after surgery, while other authors had taken it at 14 (7) to 23 (18) days after surgery. Besides this, the detection of the initial bone formation by radiologic methods (1, 20) is even later than the finding of bony spicules using the microscope.

The osteotomy and the begining of the distraction was an important stimulus for the hypertrophic reaction of the periosteum. The role of the periosteum is essential for distraction osteogenesis, as bone formation is disturbed after circumferential stripping of the periosteum (15). The bone repair response came almost exclusively from the periosteum during the first days after the operation.

Yasui *et al.* (27) documented a periosteal reaction three days after the osteotomy. However, in our study a small amount of new osseous tissue was growing under the periosteum before the distraction was initiated. Until the third or fourth week the periosteal callus was larger than the endosteal and medullary osteogenesis. At the end of the lengthening period the osteogenesis was similar in both areas. A progressive involution of the periosteal callus was seen during the resting phase. Nevertheless, 24 weeks after completion of the elongation, some signs of activity were still evident in the periosteum of the newly formed bone.

The bone marrow cavity was initially replaced by a fibrous interzone, surrounded by a bone healing response on both sides ("osteogenesis front"). After 8 weeks of neutral fixation the fatty and hematopoietic marrow cells already occupied the whole medullary canal, and the bone marrow callus had disappeared, as pointed out by Komuro *et al.* (16). The medullary reaction seems to remain active until the fusion of both neocortices. The osteogenic activity of the bone marrow is not clearly defined. During the lengthening a temporary decline in the hematopoietic function of the marrow is observed. During the fixation period following elongation, the hematopoietic cell population gradually returns to normal (13, 20). This suggests that the same stromal stem cells may be precursors for both hematopoietic and osteogenic cells (13).

Previous studies had not described the moment when the remodeling inside the distraction callus started. The remodeling of the distraction gap in our animals began very early. After the first week of lengthening the presence of osteoclasts in the newly formed bone was already seen. The osteoclastic activity was high during the whole lengthening. It is not easy to determine the remodeling time of a distraction callus. The remodeling activity in our rabbits was still present 24 weeks after the elongation was over. Costantino *et al.* (6) did not find any significant difference in the canine mandible between the area submitted to a 2.5-cm distraction and the adjacent bone one year after the lengthening had been finished. However, biopsies in humans by Shearer *et al.* (22) and Tajana *et al.* (24) showed that the remodeling of the distraction callus was not completed even 24 months after the bone elongation started.

Frierson *et al.* (9) reported that the use of an oscillating saw in distraction osteogenesis induced a delay in the consolidation in the interfragmentary gap in three of the four tibias they studied, ten weeks after distraction was over. These findings were probably a result of greater vascular damage when compared to corticotomy or osteotomy with multiple drill holes and an osteotome (9). We performed the osteotomy with a power saw, based on the analysis by Delloye *et al.* (7) who did not identify any difference in the pattern of bone healing and the amount of newly formed bone after corticotomy with a chisel or osteotomy with a driven saw. Consolidation was achieved in our rabbits between the fourth and the eighth week after elongation was stopped. The waiting period was the same (7 days) in our experiment and the study by Frierson *et al.* (9) The osteotomy was diaphyseal in all the animals. It is probable that the differences in these results could be related to the fact that Frierson *et al.* (9) used mature dogs, while we performed the distraction in immature rabbits. Although there are no series comparing osteogenic activity in lengthening in immature versus mature animals, osteogenesis in humans is slower in adults (19). The delayed elongation after surgery is an important factor in promoting osteogenesis. It seems that its effects are partially mediated by an improvement in the extraosseous blood supply (26). One week might not be sufficient to recover the blood supply on both sides of the osteotomy when using adult dogs, but it is probably enough when studying immature rabbits.

It has usually been admitted that metaphyseal osteotomy induced a greater osteogenic response in bone lengthening than diaphyseal osteotomy. Although Aronson *et al.* (4) and Fischgrund *et al.* (8) confirmed this hypothesis, Steen *et al.* (23) could not find any significant difference in relative torsional strength of the elongated tibias when comparing metaphyseal and diaphyseal bone lengthening. Metaphyseal osteotomy associated with a uniplanar external fixator is very difficult to apply in the rabbit femur. Based on successful bone lengthening in rabbits by other authors (1, 15, 17, 26, 27) when performing the osteotomy in the diaphysis, we used this surgical technique because it was safe and it permitted the easy application of a unilateral external fixator.

Previous investigations of distraction osteogenesis have pointed out that bone healing inside the callus could be accomplished by exclusively intramembranous ossification (2, 12, 18, 24) or by mixed membranous and endochondral osteogenesis, with an intramembranous (7, 9, 14, 16, 21) or endochondral (15, 26) predominance. The evolutive analysis of our histologic sections demonstrated that osteogenesis in the callus was mixed, with predominance of membranous bone formation. The presence of cartilage was always limited to the ossification front which surrounded the fibrous interzone. The cartilage finally disappeared between 5 and 9 weeks after the distraction was finished. Several previous studies have described the disappearance of cartilaginous foci from the distraction callus 4 to 5 weeks after lengthening was completed (14, 16), but Frierson *et al.* (9) found chondrocytes inside the sections until 10 weeks after the end of distraction. These findings emphasize that the cartilage formation could be caused by ischemia limited to the area surrounding the fibrous interzone, as the rapid growth of this region could be a restrictive factor for the appropriate vascularization of the whole ossification front. The decrease in local oxygen tension in some areas could be the reason why the undifferentiated cells differentiate into chondrocytes instead of osteoblasts (14, 16). The relative instability of the fixation frame could also explain the formation of cartilage foci (16, 26). Experimental conditions in animal bone lengthening (rate and frequency of distraction, waiting period, stability of external fixator, type of osteotomy, immaturity) seem to play a very important role in determining the ossification model observed in each study.

It can be concluded that the osteogenesis model of the distraction callus was of a mixed type in our study, with a predominance of intramembranous ossification. The largest part of the newly formed bone was deposited under the periosteum. The neocortex remodeling started during the first week of lengthening, and it continued 24 weeks after the end of the distraction.

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SAMENVATTING

J. MORO ROBLEDO, V. DE LA VARGA SALTO, E. GUERADO PARRA, J. DE SANTOS DE LA FUENTE, R. LOPEZ AREVALO, A. ESPEJO BAENA. Histologisch onderzoek van de distractieosteogenese van de femurdiafyse bij het imature konijn.

De auteurs hebben bij het onvolwassen konijn de distractiecallus bestudeerd. Dertig proefdieren, onderverdeeld in 6 groepen werden daarvoor gebruikt. De distractie tussen de osteotomie-uiteinden geeft aanleiding tot een bindweefselmassa, die nadien ossifieert. Vier tot 8 weken na het beëindigen van de distractie verdwijnt de bindweefselmassa. De ossificatie is van het gemengde type, membraneus en met kraakbeenvoorlopers, met voorkeur voor de eerste. De botheling in het begin van de remodellage wordt vastgesteld na één week. De originele cortex verdwijnt tijdens de verlenging en een neocortex verschijnt progressief. Deze is na 24 weken nog onvoltooid.

RÉSUMÉ

J. MORO ROBLEDO, V. DE LA VARGA SALTO, E. GUERADO PARRA, J. DE SANTOS DE LA FUENTE, R. LOPEZ AREVALO, A. ESPEJO BAENA. Etude histologique de l'ostéogenèse en distraction au niveau du fémur du lapin.

Les auteurs ont réalisé une étude de la zone d'allongement sur le fémur du lapin en croissance. L'étude a porté sur 30 animaux, répartis en dix groupes. La distraction entre les deux extrémités d'une ostéotomie fémorale a entraîné la formation d'une masse conjonctive qui s'est ossifiée ultérieurement. Cette masse conjonctive a disparu entre la quatrième et la huitième semaine qui ont suivi la fin de l'allongement. L'ossification est de type mixte, endomembranaire avec stade cartilagineux, avec prédominance de la première. On a observé un régénérat osseux avec un début de remodelage en son sein, à la fin de la première semaine de la distraction. Le cortex original s'est résorbé pendant l'allongement et a été remplacé progressivement par un néocortex, dont la formation et le remodelage n'étaient pas encore terminés vingt-quatre semaines après la fin de l'allongement. Les conditions expérimentales dans lesquelles a été réalisée cette étude pourraient expliquer certaines particularités de nos observations.