Effect of distraction frequency on bone formation during bone lengthening

A study in chickens

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ABSTRACT We compared the effects of two distraction frequencies on bone formation during tibial lengthening by evaluating radiographs, bone mineral density, and histological findings. In 15 mature White Leghorn chickens, both tibiae were distracted at a rate of 0.75 mm/day for 10 days. The distraction frequency was 2 steps (0.375 mm/12 hour) by hand on the right side and 120 steps (0.00625 mm/12 min) by autodistractor on the left. Serial radiographs showed faster bone formation on the 120-step side than on the 2-step side. Bone mineral density on the 120-step side was also higher than that on the 2-step side at all times. On the 2-step side, endochondral ossification was marked in the early stage of distraction; then intramembranous ossification became the main mechanism of bone formation. On the 120-step side, however, intramembranous bone formation predominated throughout the study. Our findings support the contention that, at least in skeletally mature chickens, an increase in the distraction frequency improves osteogenesis during bone lengthening.

Bone formation during bone lengthening by gradual distraction depends on various factors, such as the degree of preservation of the periosteum at the osteotomy site, the stability of the external fixation, and the timing and rate of distraction. However, only limited information is available as to the way in which the distraction frequency affects bone formation. We studied the effects of two frequencies of distraction on bone formation, using a model of bone lengthening in chickens.

Animals and methods

The protocol for this research project was approved by the University Committee for Animal Experimentation. We used 15 mature White Leghorn chickens, that weighed 2.5–3.0 kg. Following the administration of ether and an intravenous injection of pentobarbital sodium (10–30 mg/kg body weight), a longitudinal skin incision was made on the lateral aspect of the tibia. The periosteum was incised longitudinally and retracted. 4 threaded 2.2-mm pins were inserted at right angles to the diaphysis, using a special pin guide. A transverse osteotomy was performed between the second and third pins, using a hand-saw. The pins were then clamped to a unilateral external fixator designed for chickens. Both tibiae were operated on in the same manner.

7 days after surgery, distraction was begun at a rate of 0.75 mm a day and continued for 10 days. On the right tibia, the distraction frequency was 2 steps a day (0.375 mm/12 h) by hand, while on the left, it was 120 steps a day (0.00625 mm/12 min) by autodistractor. This miniature device had been checked for performance and accuracy in the laboratory and in pilot studies on animals.

RadŠgraphic evaluatŠn and bone mineral analysis

In 6 arbitrarily-selected chickens, the process of new bone formation at the distraction site was evaluated on radiographs and determinations of bone mineral density (BMD), using dual-energy x-ray absorptiometry at 12 days after operation (after 5 days of distraction), at the end of distraction (17 days after operation), and then at weekly intervals until 4 weeks after the completion of distraction.

An anteroposterior radiograph of each tibia, while holding it directly on the film, was taken, focusing on the distraction site and using 40 kV (100mA) for 0.5 seconds at a distance of 80 cm from the incident source. Radiographic consistency was checked on each occasion. After that, bone mineral analysis of the distraction space was done in vivo, using dual-energy x-ray absorptiometry (DCS-600, ALOKA, Tokyo, Japan). During the administration of ether, the tibia was placed parallel to the scanning plane by holding it in a special apparatus. A 30 mm length of bone, including the distraction space, was scanned at the rate of 1 mm per second. The data were analyzed with Hamanishi et al.'s method (1994). After determining the region of interest as the proximal and distal undistracted portions (5 mm length each) and the distraction site, the mean BMD of each location was measured. The %BMD of the distraction space was calculated as the relative ratio to the averaged mean BMD of the proximal and distal undistracted portions, in order to eliminate individual differences.

Histologic evaluatŠn

3 chickens were killed by intravenous administration of pentobarbital sodium for each histological examination at 12, 17, and 24 days after operation. These times were chosen to analyze the process of bone formation in the early stage of distraction osteogenesis. Immediately after killing the chickens, both tibiae were fixed, by infusing 4% paraformaldehyde into the femoral artery, and dissected with the screws and fixator in situ. The tibiae were then fixed in 4% paraformaldehyde at 4 °C overnight, decalcified by 10% EDTA, and embedded in paraffin blocks. Sections (5 μm thick) were cut in the longitudinal plane, and stained with hematoxylin-eosin and safranin O.

Statistics

We used ANOVA to evaluate the effects of the length of the postoperative period and the daily frequency of distraction and the Mann-Whitney U-test to determine differences between sides at the same time after operation. $P < 0.05$ was considered significant.

Results

RadŠgraphic findings

Initiation of new bone formation in the distraction gap was present at 12 days after operation on the 120-step side, and at 17 days after operation on the 2-step side. On the 120-step side, the radiographs at 24 days after operation showed the first bone bridge and the distraction space filled with newly formed bone. On the 2-step side, although the first bone bridge was also seen at 24 days after operation, the distracted space was not entirely filled until 38-45 days after operation (Figure 1).

BMD

The % BMD of the distraction site on the 120-step side was significantly higher than that on the 2-step side at all times (Figure 2).

Histological findings

At 5 days of distraction, the distraction gap was filled with fibrous tissue on the 2-step side. There was a small amount of new bone formation due to endochondral ossification adjacent to the cartilage islands at the osteotomized bone ends. By contrast, on the 120-step side, new bone trabeculae oriented along the distraction force were more marked at both the proximal and distal gap ends. The histological appearance consisted mainly of intramembranous bone formation, with only sparse cartilaginous islands in the newly formed bone. At 10 days of distraction, bone formation was advanced on the 2-step side, and intramembranous bone formation predominated histologically. We still observed endochondral ossification, especially in the circumferential region. On the 120-step side, new bone formation by intramembranous ossification developed more gradually and proceeded towards the center of the gap. At 1 week after the distraction, bone formation by intramembranous ossification had developed on the 2-step side, but a fibrous zone was still seen in the center of the gap. By contrast, on the 120-step side, the gap was entirely filled by new bone tissue containing many vessels (Figure 3).

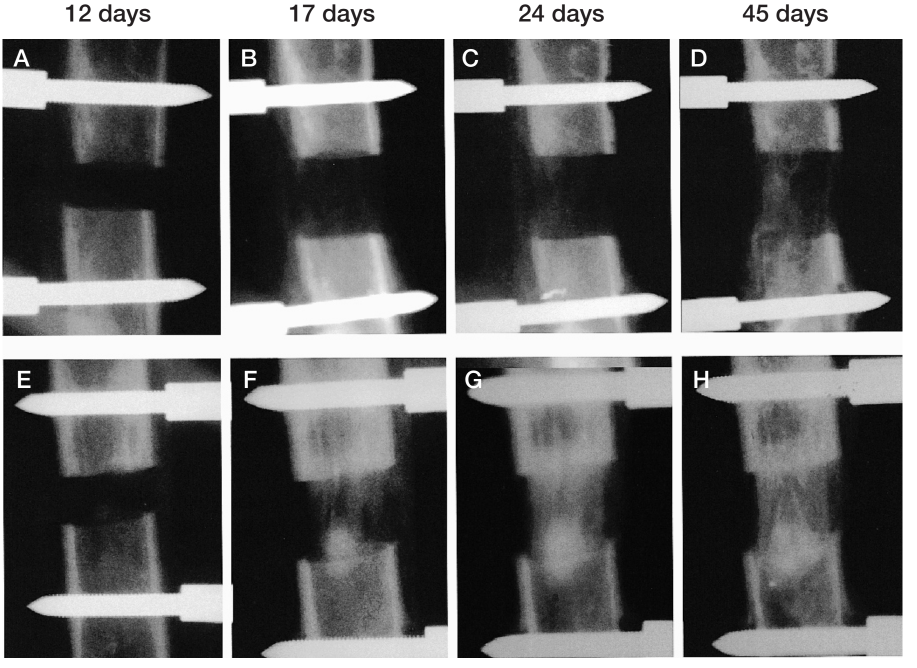

Figure 1. Typical radiographs of the lengthened tibia on the 2-step side (A to D) and on the 120-step side (E to H).

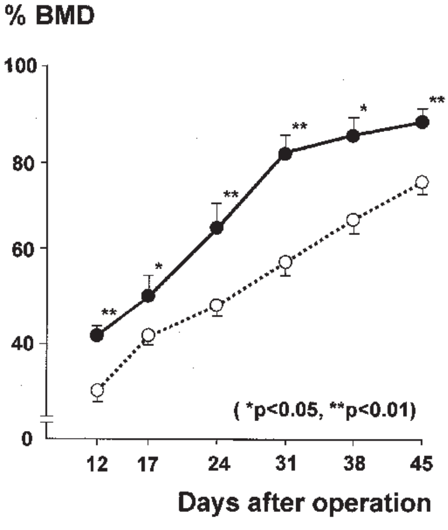

Figure 2. Bone mineral density changes in the distraction gap (mean SD) after the operation on the 2-step (❍) and 120-step sides (●).

Discussion

A few reports have explored the effects of distraction frequency on osteogenesis during bone lengthening by gradual distraction, but there is some doubt as regards the stimulation of bone formation. In early studies in a canine model, Ilizarov (1989) showed with radiographic and histochemical analyses that an increase in the frequency of distraction induced better osteogenesis. More recently, Paccione et al. (2001) have found a similar correlation between the distraction frequencies and the histological ranking scores of regenerate bone formation in a rat model of mandibular distraction osteogenesis. On the other hand, Welch et al. (1998) assessed bone formation activity during distraction osteogenesis in a goat model, using histomorphometric analysis, and found that an increase in the rhythm of distraction did not stimulate the formation of bone.

We used chickens because they are bi-pedal, and we used both legs to ensure an equal distribution of weight. Recently, Radomisli et al. (2001) showed that mechanical loading affects osteoblastic function during distraction osteogenesis and that weight bearing stimulates intramembranous ossification in the lengthened site. The bilateral use of the fixators in our study was tolerated well by the animals. They could walk without breaking step even during distraction, and they survived the entire experiment.

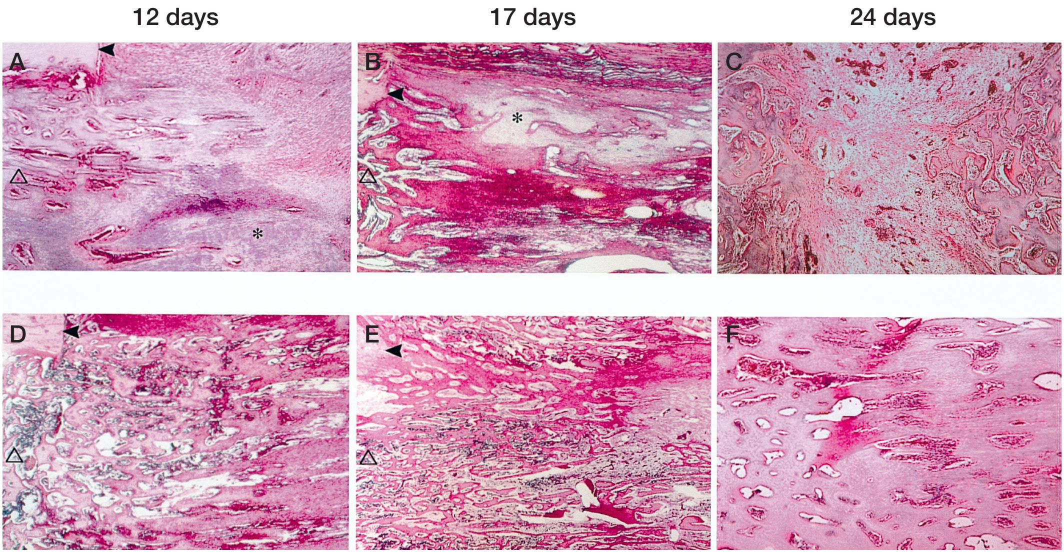

Figure 3. Typical histological longitudinal sections (HE) of the lengthened segment on the 2-step (A to C) and 120-step sides (D to F). Arrow, triangle, and asterisk indicate an osteotomized bone end, bone marrow cavity, and cartilage, respectively. C and F show the center of the gap. Magnification ×25 (A, B, D, and E) and ×40 (C and F).

Our radiographic and densitometric analyses showed that the formation of new bone in the lengthened segment was better on the 120-step side than on the 2-step one. These findings support the view that a faster frequency of distraction stimulates the formation of more bone. Like Ilizarov (1989) and Paccione et al. (2001), we used skeletally mature animals, while Welch et al. (1998) chose skeletally immature goats as their animal model. The greater osteogenic potential in skeletally immature animals may surpass the acceleratory effect produced by increasing the rhythm.

Most histological studies have shown that the main mode of bone formation during distraction osteogenesis is intramembranous ossification (Delloye et al. 1990, Karaharju et al. 1993, Aronson and Shen 1994). However, using a rat model, Yasui et al. (1997) found that the mode of ossification changed during distraction osteogenesis: endochondral bone formation was marked in the early stage of distraction, but intramembranous bone formation predominated later. In our study, the histological features on the 2-step side resembled those described by Yasui et al. (1997). New bone in the gap was formed mainly by endochondral ossification at 5 days of distraction (12 days after operation), and then predominantly by intramembranous bone formation. By contrast, on the 120-step side, intramembranous ossification was the main mechanism of bone formation throughout the study. On the basis of these findings, we believe that an increase in the distraction frequency induces intramembranous ossification at an early stage of distraction. However, too few specimens were available to be certain. Although this mechanism could not be clarified by the present study, one explanation is that a higher frequency of distraction may provide a better blood supply to the distraction site. The local blood supply has been regarded as a factor affecting the pattern of the bone-forming process: direct bone formation occurs in regions with adequate vascularity, and cartilage forms in an ischemic area (Bruder et al. 1994, Carter et al. 1998). Ilizarov (1989) found that an increase in the distraction frequency accelerated neovascularization in the paraosseous tissues of the distraction zone. A more fractionated rhythm of distraction may stimulate angiogenesis in lengthened segments, thereby promoting the direct formation of intramembranous bone. Obviously, more studies are needed to elucidate in detail the effects of the distraction frequency on osteogenesis.

In previous studies, we reported less damage to the articular cartilage in adjacent joints (Nakamura et al. 1993, 1995a, b) and to peripheral nerve function (Mizumoto et al. 1995), as well as better adaptation of muscles (Mizumoto et al. 1996), during high frequency distraction. The present study also showed that a high frequency of distraction stimulates better bone formation in skeletally mature chickens. Given the similarity of the histological changes on the 2-step side in our model and those in mammalian models, we speculate that these findings may also apply to mammalian species.

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No competing interests declared.

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