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ORIGINAL PAPER

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Distraction osteogenesis by Ilizarov and unilateral external fixators in a canine model

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Abstract We studied distraction osteogenesis in canine experimental model using two types of external fixators, Ilizarov (*n*=6) or AO unilateral (*n*=9) external fixator. Distraction started 1 week after surgery (2×0.5 mm/day) and lasted for 3 weeks. Specimens were harvested from weeks 7 through 12. The outcome was assessed by X-ray, histology, histomorphometry and microradiography. Bone regeneration as observed by X-rays was satisfactory and similar in both groups. Both endochondral ossification and intramembranous ossification were found simultaneously in both groups. In both groups, bone formation parameters were significantly higher in the area of consolidating bone. No differences in histomorphometric parameters existed between the groups. In the study period, the bone formation was enhanced and prevailed in the distraction area. This study demonstrated the utility of the canine experimental model for the study of distraction osteogenesis.

Résumé Nous avons étudié l'ostéogenèse par distraction dans un modèle expérimental canin qui utilise deux types de fixateurs externes, Ilizarov (*n*=6) ou AO unilatéral (*n*=9). La distraction a commencé 1 semaine après la chirurgie (2×0.5 mm/jour) et a duré 3 semaines. Les spécimens ont été prélevés entre la septième et la douzième semaine. L' étude a été faite par rayons-X, histologie, histomorphométrie et microradiographie. La régénération de l'os observée sur les radiographies était satisfaisante, et semblable dans les deux groupes. L'ossification enchondrale et l'ossification périostée ont été notées simultanément dans les deux groupes. Dans les deux groupes paramètres de la formation de l'os était considérablement plus haut dans la région de consolider l'os. Aucune différence dans les paramètres histomorphométriques n'a été trouvé entre les groupes. Dans la période de l'étude, la formation de l'os a été majorée et a prédominé dans la région de la distraction. Ce travail a démontré l'utilité du modèle expérimental canin pour l'étude de l'ostéogenèse dans la distraction.

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Introduction

The common principles of limb lengthening techniques are osteotomy/corticotomy and slow progressive distraction by an external fixation device. It has been established that slow distraction stimulates osteogenesis and the surrounding soft tissues. New bone is formed by endochondral or intramembranous ossification. Factors such as the stability, timing and rate of distraction, and species-related difference determine the contribution of endochondral and intramembranous ossification. Several species have been employed as experimental models for both limb and cranial distraction osteogenesis, e.g. sheep, rabbit, pig, goat, dog, mouse and rat [1, 4, 7, 10, 19, 20].

Regarding surgical technique, a transverse osteotomy resulted in regenerate bone that was indistinguishable from the bone obtained after the technically more difficult corticotomy [5]. Rate of distraction is also a critical determinant of this procedure's outcome, i.e. in the rabbit model, cell proliferation was increased at rates of more than 0.3 mm/day, while 0.7 mm/day appeared optimal for cell proliferation and histological characteristics [9].

The aim of this study was evaluation and morphological characterisation of the canine model of distraction osteogenesis by two established fixators.

Materials and methods

Animals

Fifteen 1-year-old German shepherd dogs were used. Housing, care and experimental protocol were in accordance with guidelines established by the Institutional Animal Care and Research Advisory Committee.

Operative protocol

Under general anaesthesia and aseptic technique, corticotomy of the tibia was performed, after which either Ilizarov (n=6) or AO unilateral (n=9) external fixator was applied. Distraction started 1 week after surgery (2×0.5 mm/day) and lasted 3 weeks in both groups. Consolidation period started at week 4 after surgery. Specimens were harvested from weeks 7 through 12. Fluorochrome bone labels were administered intravenously for assessment of bone formation. Bone healing was monitored by radiographs. Satisfactory stability of the bone fragments could not be achieved in all experimental animals. This was not related to the type of the fixator applied.

Histology and histomorphometry

Upon experiment termination, tibia specimens were removed, cleaned of soft tissues, fixed and prepared for undecalcified processing. On each histology slide, the bone regenerate area encompassing 2 cm or more of the corticotomy site was defined, and the identical area was used for microradiography. Within this bone area, the percentage of cartilage, fibrous tissue, trabecular and cortical bone was evaluated on Giemsa stained sections. Both static (osteoid surface [OS/BS], eroded surface [ES/BS] and osteoid thickness [OTh]) and dynamic histomorphometric parameters (mineral apposition rate [MAR] and bone formation rate [BFR]) in the area of bone regenerate and normal bone (distant from the surgery site) was performed. MAR was measured on opposite cortices distant from the regenerate area in order to detect the differences, which might have occurred due to bone bending.

On microradiographs, the percentage of calcified bone tissue was assessed within the regenerate area corresponding to the same area on histological slides. Histomorphometric analysis was carried out using a semiautomatic image analysis system (VAMS, Zagreb, Croatia) at ×40 magnification. The nomenclature and calculations were in accordance with the American Society of Bone and Mineral Research Histomorphometry Nomenclature Committee.

Results

Radiographs

Monitoring by X-rays showed that the final outcome was similar and satisfactory for both groups (Figs. 1 and 2). Poor alignment and fragment retention occurred in three cases resulting in a non-union and delayed fracture healing.

Histology

Histology revealed similar ossification processes in both groups. Endochondral ossification (Fig. 3), originating from the cortices, and intramembranous ossification (Fig. 4) were found simultaneously. Endochondral ossification remained mostly as cartilaginous collar adjacent to cortices and periosteal tissue during the histological follow-up of the consolidation. Much lesser amounts of cartilage and its residues in an irregular jagged line could be observed as interface between the distracted frag-

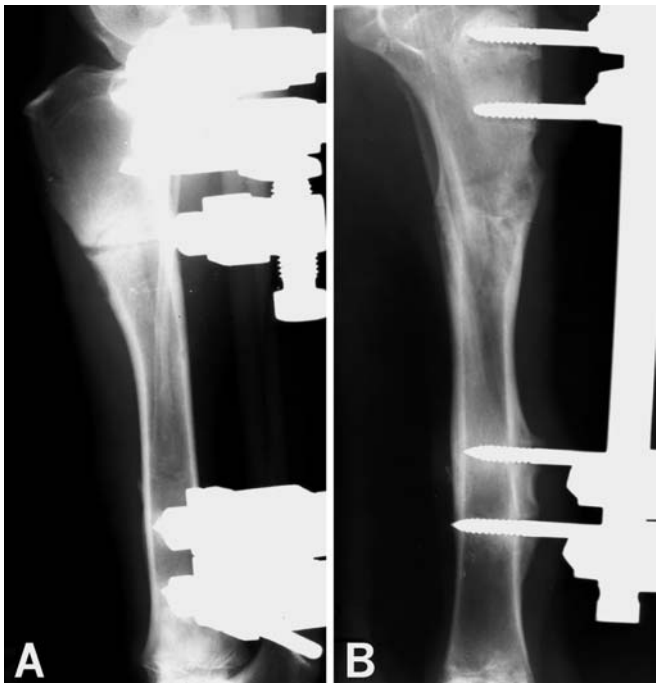

Fig. 1 Radiographs of the distraction area at 2 (*A*) and 12 (*B*) weeks following surgery showing pronounced osteogenesis and good alignment of the fragments in a case with the AO unilateral external fixator

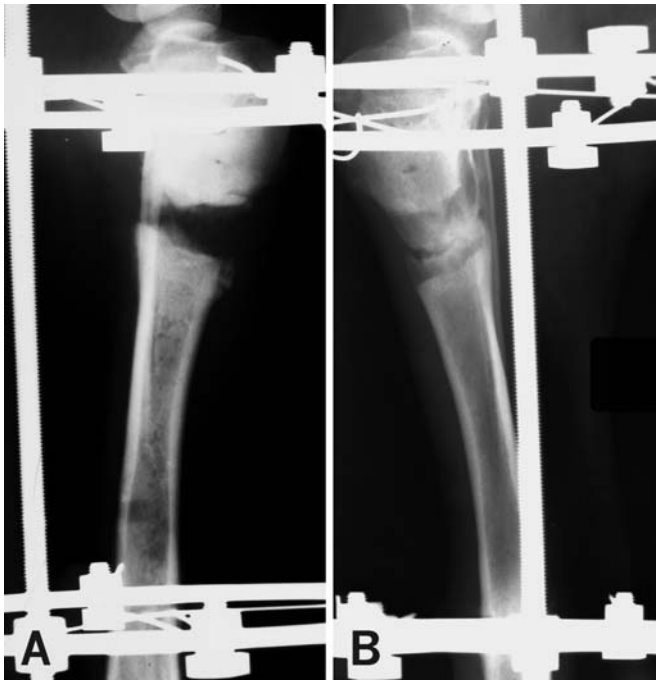

Fig. 2 Radiographs of the distraction area at 2 (*A*) and 12 (*B*) weeks following surgery showing vivid osteogenesis and calcification of the callus in a case with the Ilizarov external fixator

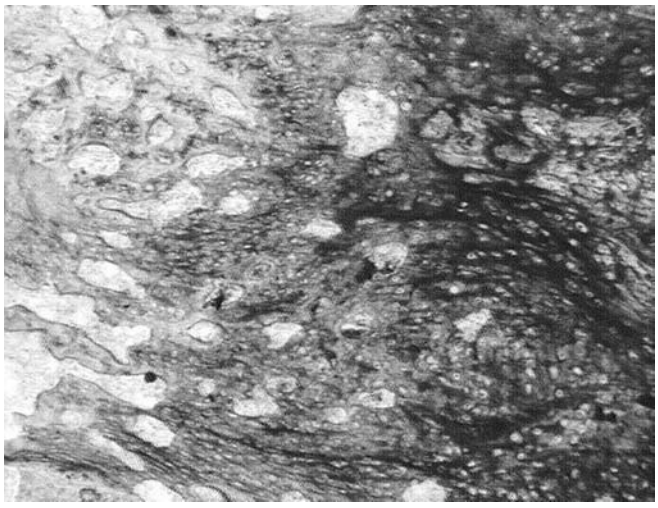

Fig. 3 Endochondral ossification arising mostly from the cortices of the bone fragments. Hyaline cartilage and mineralised cartilage were found simultaneously with newly formed bone (Giemsa staining, magnification ×40)

Fig. 4 Intramembranous ossification occurred mostly in the central part of the regenerate area. Osteoblasts on the surface of the newly formed bone surrounded by fibrous tissue from which the osteoblasts differentiated (Giemsa staining, magnification ×40)

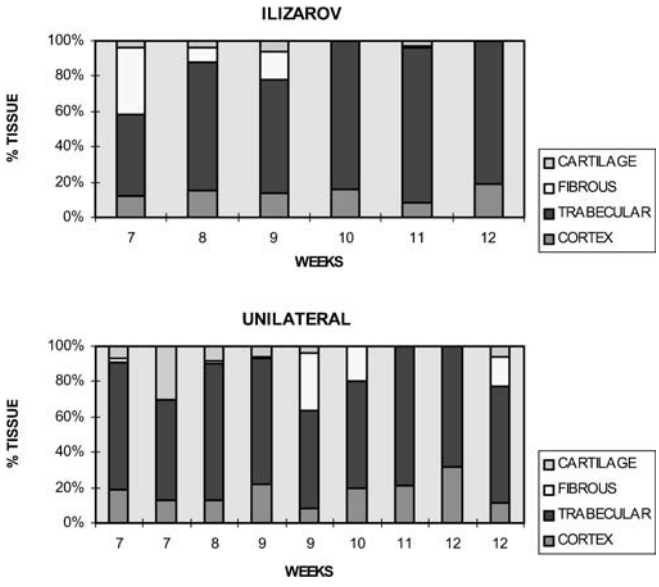

Fig. 5 Distribution of tissues (percentage) assessed in the regenerate area of the two groups

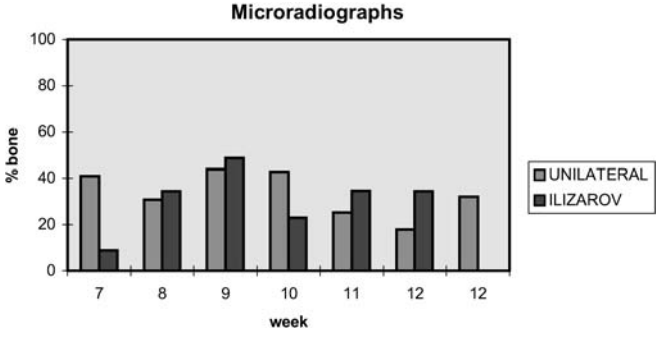

Fig. 6 Percentage of mineralised bone measured in the regenerate area as seen on microradiographs

ments. Regeneration progressed centripetally from the cortices, with cartilage and calcified cartilage as a template prior to the consolidation period. Intramembranous bone formation predominated within the medullary part of the distraction gap. Columns of bone tissue and interconnecting trabeculae were oriented longitudinally in the direction of distraction. Proportions of tissues in the regenerate area for the Ilizarov fixator showed a trend of increment for trabecular bone, diminution of fibrous tissue and a continuous small percentage of cartilage located at the external collar (Fig. 5). For the unilateral external fixator group, no clear trend could be observed for different tissue percentages during the same period. Considerable variation existed between two experimental animals of the unilateral fixator group for weeks 7, 9 and 12 of the consolidation phase, due to differences in the success of fixator application.

Microradiographs

Bone regenerate originated from the dissected cortices and usually included periosteal bony reaction. The degree of subperiosteal osteogenesis varied considerably between experimental animals. Longitudinally oriented bony spicula and trabeculae progressed towards the middle part of the initial distraction gap. Towards the end of the experiment, only a narrow irregular gap remained or was completely bridged. Successful healing revealed on microradiographs more trabecular bone in the regenerate area than in other parts of the diaphysis. Percentage of mineralised bone evaluated on microradiographs in the regenerate zone varied between 9 and 49% for the Ilizarov and between18 and 44% for the unilateral fixator group (Fig. 6).

Table 1 Histomorphometric data for the Ilizarov and unilateral external fixator for the regenerate zone and normal bone. *OS/BS* osteoid surface (percent), *ES/BS* eroded surface (percent), *OTh* osteoid thickness (micrometres), *MAR* mineral apposition rate (micrometres per day), *BFR* bone formation rate (micrometres per day)

Week	Remodelling zone					Normal bone				
	OS/BS	ES/BS	OTh	MAR	BFR	OS/BS	ES/BS	OTh	MAR	BFR
Ilizarov										
7	6.5	9.6	17.5	1.24	0.21	1.3	7.0	11.8	1.00	0.14
8	16.2	20.0	17.2	1.56	0.34	10.0	5.1	15.7	1.37	0.26
9	26.6	3.8	12.8	1.36	0.26	3.0	6.8	14.4	1.06	0.16
10	50.4	2.7	25.5	1.43	0.29	34.5	6.2	16.3	1.11	0.17
11	34.7	9.8	17.1	1.43	0.29	24.8	8.6	17.9	1.47	0.30
12	66.0	4.6	14.3	1.58	0.35	25.6	13.5	11.0	1.36	0.26
Unilateral										
7	31.8	7.0	10.0	1.34	0.25	10.1	8.4	9.0	1.64	0.38
7	35.0	14.6	11.6	1.79	0.45	13.7	8.7	14.1	1.57	0.35
8	41.9	5.1	10.2	1.43	0.29	39.5	10.1	10.1	1.32	0.24
9	39.5	3.9	11.3	1.84	0.48	31.0	14.8	12.8	1.50	0.32
9	24.5	1.6	19.5	1.51	0.32	15.4	1.7	11.7	1.26	0.22
10	38.3	5.3	17.0	1.21	0.21	30.1	14.1	8.1	1.07	0.16
11	28.6	5.6	14.9	2.50	0.88	37.8	4.0	11.1	1.38	0.27
12	51.3	2.9	17.2	1.75	0.43	13.8	0.0	9.9	1.44	0.29
12	26.6	10.2	14.5	2.69	1.01	33.8	9.4	11.9	2.16	0.65

Histomorphometry

Histomorphometric data are presented in Table 1. Assessment of proportions of tissue types in the regenerate area showed an increase in the amount of trabecular bone from weeks 7 through 9 and a decrease in fibrous tissue. Variations beyond week 9 were less pronounced. Percentage of cartilage in this area was small and constant from weeks 7 through 9 and almost diminished thereafter. In the experiment with the unilateral fixator, two experimental animals were available for weeks 7, 9 and 12, contributing to the overall variation. Percentages of trabecular bone were 60% and more throughout the experiment but lacked a clear trend of increase due to unsatisfactory stability of the fragments. Fibrous tissue showed higher percentage in these cases.

Static parameters showed greater variation than dynamic parameters. Comparison of the entire data for the Ilizarov and unilateral fixator group, as tested by Wilcoxon test, showed no difference.

Data on both fixators were pooled and tested (Wilcoxon paired test) between the regenerate zone and normal bone, and showed significantly higher values in the regenerate zone for OS/BS, MAR, BFR (all $P<0.01$) and OTh ($P<0.05$). MAR also was determined on two opposing cortices in the normal bone area and the differences tested. Proportions of statistically significant differences figured 1/6 (17%) for the Ilizarov fixator and 4/11 (44%) for the unilateral fixator. These proportions did not differ significantly between the two groups.

Discussion

The canine experimental model of distraction osteogenesis achieved by two types of fixators, i.e. the Ilizarov and unilateral types, was satisfactory and similar in outcome in the follow-up by X-rays. In another study, equal bone volume was found by Ilizarov and Wagner fixators despite a significant difference in axial rigidity [1]. Meffert et al. [13], using the rabbit long bone model, stressed the importance of evaluating the experimental model, i.e. the type of external fixation system and fixation technique, when designing experiments.

A similar progress in ossification of the distraction gap between the two groups was confirmed by histology. Intramembranous ossification predominated in the healing area occupying most of the distraction gap between the dissected diaphyseal cortices. It might be speculated that the early consolidation phase, i.e. before week 7, might have revealed more endochondral ossification than the latter consolidation phase, which was studied in this experiment. Sato et al. [17] described histological characteristics during distraction in rats and found that cartilage was progressively resorbed from both ends and new bone was formed directly by intramembranous ossification. In rat, the distraction produces an environment in the distraction gap that suppresses the formation of cartilage. Furthermore, the formation of cartilage by injured periosteum is obligatory and does not appear to be influenced by distraction [6]. Li et al. [11] have observed overlapping cartilage-bone phenotype (collagen types I and II) in cells of the cartilage-bone transitional region in endochondral ossification in rabbits, proposing the hypothesis that the hypertrophic chondrocytes may transdifferentiate into bone cells. Three modes of ossification were reported in the rat by Yasui et al. [21] i.e. chondroid bone besides the endochondral and intramembranous type. Kojimoto [7] stressed the important role of the periosteum as its removal caused failure of callus formation and bone lengthening. However, no differ-

ences were found in the pattern of bone healing and the amount of newly formed bone after corticotomy or osteotomy [2].

On microradiographs the percentage of mineralised bone was analysed in the identical area evaluated for histology. Variations of the amount of mineralised bone within each fixator groups and also between the groups existed. In comparisons of proportions of trabecular bone stained by Giemsa, it was obvious that non-mineralised and mineralised bone could not be distinguished by that method, stressing the advantages of applying several methods when evaluating distraction osteogenesis in the canine model. Smith et al. [18] applied computed tomography for this purpose.

The underlying molecular mechanism of bone formation during distraction osteogenesis was ascribed to enhanced expression of BMP-2 and BMP-4 genes induced by mechanical tension-stress [10, 17]. The presence in the distracted region of insulin-like growth factor (IGF)-1, transforming growth factor (TGF)-β and basic fibroblast growth factor (FGF)-1 may account for the proliferation of osteoblast and its formation from precursor mesenchymal cells. All factors were induced by mechanical strain [4, 8]. TGF-β, its receptor and IGF-1 also were found in the human callus during callostasis [3]. Accelerated ossification of bone regenerate in distraction osteogenesis can be achieved by systemic administration of recombinant homologous growth hormone [15].

Histomorphometric data confirmed intensive formation in the regenerate area, where bone surface with osteoid and osteoid thickness were significantly higher. MAR and BFR in our study were significantly greater in the regenerate area compared to other bone areas in both fixator groups. Mehrara et al. [14] showed that osteoblast differentiation and matrix synthesis coincide with TGF-β gene and protein production and osteocalcin gene expression with matrix mineralisation. Degree of mineralisation has been related to osteocalcin production [12]. We found no relationship between histomorphometric parameters and time in the consolidation period, suggesting that formation processes were not yet slowing down. It has been demonstrated that the enhanced bone formation and remodelling results more from increased recruitment and activation of bone-forming and -resorbing cells rather than from an increased level of individual cellular activity [20]. No difference in the extent of eroded surface existed between the regenerate area and other bone areas. This is also in agreement with the previous observation that during this part of the consolidation period bone formation is predominant. It can be expected that had the experiment continued more pronounced bone resorption would produce marrow cavity within the distraction osteogenesis area. Comparison of data for the two fixators also showed no statistical difference. In a mouse model, it was demonstrated that osteoclasts remodelled the bone regenerate as it formed [19]. During distraction, a wide variety of cells express the bone matrix proteins mRNA at a much greater rate than during normal fracture healing or in the growing foetal bone [16].

MAR measured on opposing cortices outside the regenerate area did not indicate the existence of differences that might have suggested bending caused by the fixator, This factor emphasises uniformity of both fixators.

In conclusion, the canine distraction osteogenesis experimental model proved to be a useful and established model for the investigation of many aspects of this corrective procedure. As assessed by X-ray, histology, histomorphometry and microradiographs, both the Ilizarov and unilateral external fixators were found similar in performance and outcome. In the study period of 7–12 weeks postoperatively, bone formation was enhanced and prevailed in the distraction area.

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