ORIGINAL ARTICLE

Effects of osteoformin in the rapid distraction osteogenesis of rabbit mandibles

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Abstract

Objective: To evaluate the effects of osteoformin on mineralisation and quality of the new bone formation during rapid distraction osteogenesis.

Methods: This multi-centre study was conducted at the Karadeniz Technical University, Middle East Technical University and Selcuk University. The experimental study was conducted from January 2010 to September 2012 and comprised New-Zealand rabbits that were randomly divided into three groups. In group I distraction rate was 1 mm/day while in groups II and III distraction rates were 2mm/day and 1 mm/day. In groups I and II 100µg/kg osteoformin was injected after the latency period. Distraction region was evaluated by radiological, histomorphometrical and dual energy X-ray absorptiometry analyses. SPSS 17 was used for statistical analysis.

Results: There were 18 rabbits with each of three groups having 6(33.3%). Accelerated bone healing was noted in groups I and II compared with group III ($p<0.05$). No significant differences were indicated between groups I and II ($p>0.05$).

Conclusion: Local injection of osteoformin was effective in the craniomaxillofacial distraction osteogenesis in rabbits. Further experimental studies are recommended before using osteoformin on humans.

Keywords: Distraction osteogenesis, Bone healing, Negatively-charged peptide, Distraction rate, Animal model. (JPMA 66: 135; 2016)

Introduction

Distraction osteogenesis (DO) is a widely used procedure in oral and maxillofacial deformities. Although its principles in orthopaedic surgery have been successfully applied, the rate, rhythm and consolidation period are common problems for craniomaxillofacial DO.[1,2] Prolonged distraction and consolidation period may lead to some other problems such as pin tract-soft tissue or bone infection, patient discomfort and related social problems.[2] Shortening the consolidation period via accelerated bone formation is one of the most emphasised points in DO. For this purpose agents such as zoledronic acid, electric and ultrasound stimulation, matrix metalloproteinase-1, bone morphogenetic protein-2 and hyperbaric oxygen with different rate and rhythm protocols have been investigated.[3-7]

In the healing of fractured bones, electrical environment surrounding the injured area is an evident fact. Earlier studies showed negatively-charged resins increased bone formation whereas positively-charged resins had negative response to osteogenesis with inflammatory and fibroblastic activity[4,8,9] Osteoformin (Sigma Chemical Co., St. Louis MO), a polymer polyaspartate, is one of the negatively-charged resins. Osteoformin has been suggested to decrease healing time with improved bone quality with increased alkaline phosphotase activity and mineralisation.[10] Osteoformin, which was claimed to accelerate healing in femoral fractures, was also administered successfully to the craniomaxillofacial DO. In a previous in vivo study it was found that osteoformin increased maturation and healing rate of the new bone formation during DO in the craniomaxillofacial region.[11]

The present study was planned to investigate the behaviour of osteoformin during rapid DO, which was proven to increase maturation and healing rate during bone lengthening in mandible.

Materials and Methods

The experimental study was conducted from January 2010 to September 2012 with the ethical approval of Karadeniz Technical University Animal Research Ethics Committee. This multi-centre study was conducted at the Karadeniz Technical University, Middle East Technical University and Selcuk University. It comprised male 20-week-old New Zealand white rabbits, each weighting between 2.2 and 3.2kg, that were randomly divided into three experimental groups. The surgical procedure was performed in line with a previous study.[12] All animals were administered intramuscular IM) penicillin G for the postoperative 5 days, given a soft diet and water ad libitum, and kept alone in single cages during the study.

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In group I distraction rate was 1mm/day, while in groups II and III distraction rates were 2mm/day and 1mm/day respectively. After a latency period of 5 days, in groups I and III the left mandibles were lengthened 0.5mm every 12 hours for 10days. In group II the left mandibles were lengthened 1.0 mm every 12 hours for 5 days. In groups I and II, 100µg/kg osteoformin in 100µL/kg phosphate-buffered saline (PBS) was injected into the central zone of distraction gap at the 1st and 7th days after the latency period as described in literature.[9,10] In group III only 100µL/kg PBS was injected on the same days. The distraction apparatus was kept 42 days for consolidation. On postoperative day 42, the animals were sacrificed with high dose of sodium pentothal injection. The mandibles were dissected and placed in 10% buffered formalin solution.

Plain radiographs were taken using Trophy X-ray unit (Kodak Co. France). Specimens were placed on an occlusal film, and X-ray was directed from the buccal side of the mandibles. Radiography procedure was performed under a standard condition of 3.2-mA and a tube-to-film distance of 10cm. All radiographic evaluations were performed by the same blinded radiologist. Sections were examined for the union or non-union in the field of distraction gap.

New bone formation was evaluated by 0-2+ scale as follows; (0): Non- union of the specimen;(1): Bone formation less than 30% of the specimen; and (2): Bone formation more than 30% of the specimen.

After radiological examination the specimens were evaluated using Dual Energy X-ray Absorptiometry (DEXA) to determine bone mineral density (BMD; g) and bone mineral content (BMC;g/cm^2) values in the distraction area. Lunar DPX-IQ (Digital Picture Exchange - Image Quality) device (Madison, WI) equipped with small animal software used for measurement of DEXA. The appendicular programme was used and the measurements were performed in 0.032mm^2 space in the distraction gap.

Following DEXA measurements of the distraction gaps, specimes were fixed in 10% buffered paraformaldehyde for 48 hours and decalcified in ethylenediaminetetraacetic acid (EDTA) solution. Tissue specimens were prepared in an autotechnicon, embedded in paraffin and sectioned with microtome. The sections (5µm) were stained with Haematoxylin & Eosin (H&E). Stained specimens were investigated by a Nikon Eclipse E400 (Nikon, Tokyo, Japan) light microscope. For each specimen, the same area was photographed after staining by using a Nikon Coolpix 5000 photograph attachment. The photograph of Nikon micrometer microscope slide was also taken during the procedure. All photographs were then transferred into computer environment and analysed using Clemex Vision Lite 3.5 Image Analysis programme (Clemex Technologies, Longueuil, Quebec, Canada). The length was calibrated by comparing the photograph of specimen with the photograph of Nikon micrometer microscope slide, which was taken under the same magnification. Areas of 7925744.4 mm^2 (square micrometre) areas was designated with using Clemex Vision Lite 3.5 Image Analysis programme. Osteoblasts, osteoclasts, capillaries, fibroblasts and collagen fibres were marked with the same image analysis programme in 1981436.1 mm^2 area. New bone forming area and cartilaginous area were measured, but damaged cells were not evaluated. The marked cells were counted automatically with the same image analysis programme. The reader was masked to the origin of the specimen.

SPSS 17 was used for the statistical analysis. Kruskal-Wallis analysis followed by Mann Whitney U test with Bonferroni correction were applied.

Results

Initially 21 rabbits were included, but 3(14%) were excluded before the end of the latency period. Of them 2(66%) removed the pins of the distractors, and 1(33.3%) rabbit underwent unexpected weight loss. Groups I, II and III lost 1(14%) rabbit each, leaving a total of 18(86%) rabbits each with a mean weight 2.6±0.23 kg for

Table-1: Radiological evaluation of bone formation in distraction gaps.

	0	1	2
Group I	0%	16.6%^{A}	83.3%^{C}
Group II	0%	33.3%^{A}	66.6%^{C}
Group III	33.3%	50%^{B}	16.6%^{D}

*In each column different superscript capital letters indicate significant differences between the groups ($p<0.05$).

Table-2: Mean values of BMC and BMD in the distraction gap with DEXA.

	BMC (g/cm^2)	BMD (g)
Group I	0,008±0,001^A	0,264±0,025^A
Group II	0,009 ±0,001^A	0,287±0,037^A
Group III	0,005 ±0,000^B	0,156±0,018^B

*In each column different superscript capital letters indicate significant differences between the groups ($p<0,05$).
DEXA: Dual Energy X-ray Absorptiometry
BMC: Bone mineral content
BMD: Bone mineral density.

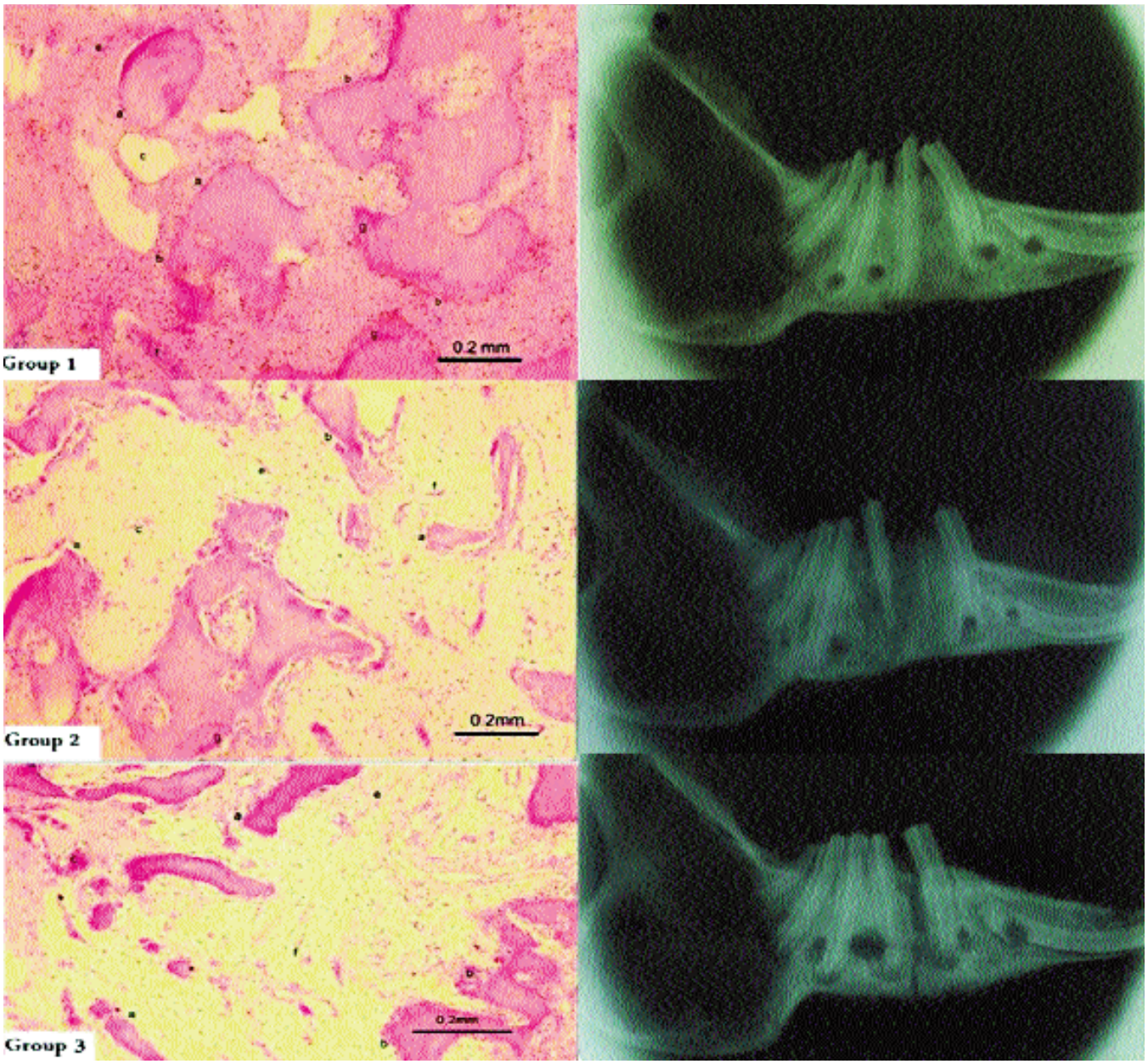

Figure: Histological (left) and radiological (right) view of the specimens. Note the newly-formed bone area in groups (Haematoxylin-eosin) a: Osteoblast b: Osteoclast c: Vessel e: Fibroblast f: Kollagen g: Newly-formed bone.

evaluation, with each of the three groups having 6(33.3%) animals. All rabbits recovered uneventfully from surgery and none experienced post-operative complications, and distractors were stable until sacrifise. Obvious crossbite and overgrowth of the lower incisor developed in all rabbits.

A statistically significant difference between the groups for new bone formation was observed during radiological evaluation (p=0.037). Increased bone formation was noted in groups I and II compared with group III (p= 0.004 and p=0.01). On the other hand, intergroup analysis indicated no significant differences between the groups I and II (p=0.523) (Table-1). Evident osteoblastic activity with new bone formation was noted in groups I and II compared with group III (Figure).

A statistically significant difference between the groups

Table-3: Mean values of newly-formed bone area, vessels, number of osteoblasts, osteoclasts, collagen fibres and fibroblasts in the distraction area.

	Group I	Group II	Group III
Newly formed bone area (mm^2)	451641.3±173276.7^A	398558.0±171769,3^A	286984.5±215506.5^B
Vessels	18.2±4.1^A	16.8±5,3^A	14.6±5.4^B
Osteoblasts	63.1±22.5^A	51.4±12,6^A	37.6±4.2^B
Osteoclasts	8.7±0.8	8.2±4,1	7.5±2.5
Collagen fibers	14.9±3.8^A	14.6±14,9^A	12.3±7.3^B
Fibroblasts	36.5±13.8^A	34.2±3,9^A	31.0±6.9^B

*In each line different superscript capital letters indicate signficant differences between the groups (p<0,05). The mean difference is significant at the <0.05 level.

regarding BMC and BMD values were observed during DEXA analysis (p=0.043). Increased BMC and BMD values were noted in groups I and II compared with group III (p= 0.036 and p= 0.043). Intergroup analysis indicated no significant differences between groups I and II (p=0.152) (Table-2).

Histomorphometrical analysis comparing bone formation, number of vessels, number of osteoblasts and number of collagen fibres and fibroblasts showed that groups I and II had significantly greater values than group III (p=0.002; p=0.033; p=0.042; p=0.020; p=0.005, respectively). Intergroup analysis indicated no significant differences between groups I and II (p>0.05). No significant differences were noted between the groups regarding the number of osteoclasts (p=0.054) (Table-3).

Discussion

The application of DO for adjusting congenital deformities occurring in craniomaxillofacial area became a popular technique and many operations have been performed all around the world.[12,13] The reduced necessity of bone grafting and donor-site morbidity and the concomitant adaptation of the soft-tissue, called distraction histogenesis, are the main advantages of craniomaxillofacial DO.[14] The main disadvantage of DO in the craniofacial skeleton is the long distraction and consolidation periods. The latency, rate, rhythm and consolidation periods are common problems because of prolonged distraction time.[2,3] In addition, the risk of injury, fractures in the regenerated bone, bony union with fibrous callus formation and even non-union and associated social problems complicate the application of DO.[15-17]

Rapid DO would attenuate the complications associated with the overall treatment time. The intramembranous bones of the craniofacial skeleton, which has a generous blood flow, may permit the rapid distraction procedure.[18] Different rate and rhythm protocols were investigated for rapid distraction, but poor ossification, fibrous or non-union of the fragments were noted in earlier experimental studies, therefore adjunctive agents and techniques have been used for accelerated DO in order to reduce the time required for optimum consolidation.[3-7,19]

The negatively-charged dextran beads have been successfully used for critical-sized defects in the craniofacial area. The negatively-charged beads were found to have bone formation stimulating capacity whereas positively-charged beads were non-osteogenic.[8,9] Osteoformin was suggested to accelerate the rate of fracture healing in a closed femoral fracture.[10] In a study, osteoformin was administered to the craniomaxillofacial DO. It was shown that osteoformin significantly increased maturation and healing rate of new bone formation in rabbits during DO in the mandible. One study noted that osteoformin significantly increased BMD and BMC values in distraction region.[11] In the present study, although there were no significant differences observed between groups I and II, increased BMC and BMD values were noted compared with group III which was consistent with the literature.[11] Histomorphometric results also supported DEXA results; an increased number of the -formed bone area, number of vessels, osteoblasts, collagen fibres and fibroblasts were noted in groups I and II compared with group III. However, there were no significant differences between the groups regarding the number of osteoclasts.

In the present study, osteoformin increased mineralisation and quality of the regenerate in the time course of consolidation. Although radiological analysis indicated no significant differences between groups I and II, but increased bone formation was noted in groups I and II compared with group III. In addition, obvious radio-opacity was noted across the distraction gap in groups I and II, whereas incomplete union at the central zone of the distraction regenerate was noted in group III. Therefore, adjunctive osteoformin injection may be effective in reducing overall treatment time by increasing the resistance of the regenerated bone in DO.

The majority of the complications in DO are related to long duration of therapy, therefore increased efforts were made to shorten the treatment with accelerated bone healing. Adjunctive therapies in DO are usually intended to shorten the duration of consolidation by increasing resistance of the regenerate. Osteoformin was previously suggested to increase BMC and BMD values at a rate of 1mm/day distraction.[11] In our study, osteoformin

improved healing of regenerate at a rate of 2mm/day distraction that also shortened the duration of distraction period. Prolonged distraction and consolidation periods may result in the failure of the region of regenerate as a result of non-function atrophy, increased risk of infection and osteoporosis in the surrounding bone caused by leaving the external fixator in place for a long time and functional and psychological problems.[20] Therefore, osteoformin may encourage the rapid DO protocol with accelerated bone healing.

Conclusion

Histomorphometrical analysis and DEXA results indicated that local injection of osteoformin during DO was effective in the craniomaxillofacial region in rabbits. DEXA results also supported the idea that the administration of osteoformin may allow 2mm/day instead of 1mm/day elongation. Further experimental studies are needed to be conducted before using osteoformin on humans.

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