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Effect of Fluoride on the Healing Gap Tissues in Mandibular Osteogenesis

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ABSTRACT

The purpose of this study was to measure histomorphometric bone fill after different latency periods, rates of distraction, and duration of neutral fixation in the adult goat mandible. In addition, the relationship between histomorphometric bone fill and clinical stability was investigated. Mandibular osteotomies in 18 female's adult goat weighing 15-20 kg were distracted with modified semiburied distraction devices. Animal model divided into 3 groups; GO received plain tap water, GI; received fluoridated water at the level of 10 mg/kg body weight, G2; received fluoridated water at the level of 20 mg/kg body weight. The device was activated at a rate of 1mm/day for 10 consecutive days. Total distraction distance was 10 mm, period lasted for 25 days after end of the activation period and experimental period was 40 days.

KEYWORDS: Distraction osteogenesis, goat mandible, fluoridated water, histomor-phometric

INTRODUCTION

The concepts in craniofacial and maxillofacial surgery have evolved rapidly during recent decades and now offer new techniques related to the correction of some deformities that were almost impossible to treat in the past. Distraction osteogenesis (DO) is a surgical process involving new bone formation between vascularized bone surfaces following osteomy or corticotomy as a result of regular distraction achieved by means of functionally stable devices. It is a surgical process for reconstruction of skeletal deformities.

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It involves gradual, controlled displacement of surgically created fractures which results in simultaneous expansion of soft tissue and bone. Unique feature of distraction technique is that bone regeneration by distraction osteogenesis is accompanied by simultaneous expansion of soft tissue matrix as an adaptive change to the tension generated by distraction forces '". DO advanced the field of maxillofacial surgery because of its versatility, simplicity, and possibility of avoiding bone grafts, infections, blood transfusions, or inter-maxillary fixation for long periods of time and established the augmentation of the soft tissue simultaneously with the bone. Therefor DO could be good alternative ways in many cases where conventional surgical techniques are rather difficult to use? The reliability of this method has been examined by several authors who introduced this therapeutic principle to craniofacial surgery; both experimentally and clinically to displace portion of the facial skull or the jaws (3). It is widely believed that new bone formation, occurs more or less, in same way as intermembraneous ossification. Stretching of soft tissue is possible by distraction ontogenesis without any problems at a distraction rate of one millimeter per day. All methods mentioned allow callus distraction in only one direction which is determined by the device used, however, the possibility for multidimensional distraction is still under investigations. The fluoride ion has been recognized as an important trace element for human health based upon its unique role in dental health *. Fluoride has been used extensively in the treatment of osteoporosis and has been approved by regulatory agencies in many European countries. However, its status as a therapy for osteoporosis has been controversial. Recent studies had strongly suggested that the fluoride could be the use in the treatment of osteoporosis (57). Fluorides were reported to stimulate the osteoblasts to form new bone tissue[(8-10)] . Moreover, fluorides alone and in combination with 1,25-Dihydroxyvitamin D3 prevented the loss of cancellous bone, reduced the resorption, protected trabecular micro architecture and stimulated bone formation in ovariectomized rats"). The sodium fluoride led to accelerated chondrogenesis process in the area of insufficiently perfused bone, osteogenesis including temporary callus formation and mineralization of the new bone, as well as remodelling into mature lamellar bone. He concluded that his results could be the base of clinical studies on application of sodium fluoride in the acceleration of fracture healing(12. The present study was aimed to evaluate the effect of systemic fluorides on the biomechanical, mineralization and histological properties of caprine mandibular model rapidly lengthened by distraction osteogenesis.

MATERIAL AND METHODS

1. Sample size and study population

Eighteen young adult healthy female goats of comparable age (12-16 months), weighing between 20-25 Kg was served as the experimental animals in this study. The experimental animals were divided into three groups; each group consist six goats as following: Group 0 represented control group and received just plain tap water, group I and II represented study group where group I received fluoridated water at concentration of 10 mg/kg body weight and it was considered low dose and group II received fluoridated water at concentration of 20mg/kg body weight and it was considered high dose.

2. Osteotomy process

In all animals included study, the osteotomy was created in the mandibular premolar region bilaterally. The used distractor device was Egyptian-made titanium extra-oral distractor, the surgical procedure was performed under intravenous injection of sodium thiopental 10mg/kg, and isoflurane 2% vaporized in oxygen through endotracheal intubations to maintain the airway. Betadine was used to scrub the operative site and the site of the operation was isolated and draped in the regular surgical manner. After injection of vasoconstrictor agent, an extraoral submandibular incision was made. The flap was reflected and the distraction device was adapted for a passive fit.

The edentulous premolar area of the mandible was osteomatized to create a vertical osteotomy under copious saline irrigation. The distraction device was then fixed to the bone by six self-tapping screws (9 mm length, 2 mm diameter). Two screws were fixed at both sides of the osteotomy line to calculate the actual distracted distance. The distance between these screws was measured and recorded, then the flap was sutured with 3/0 silk suture, keeping the activating rod of the distraction device protruding from the skin to activate the device daily. Same procedure was performed on the both side of the mandible Figure 1). All animals received acupan (Nefopem vial 20 mg). 1 ml intramuscularly every 12 h for 3 days to control pain and also received Ibiamox 500 mg/12h for five successive days postoperatively to control infection. Teeth and area of surgery were cleaned using tooth brush and irrigated with saline solution daily. Tribotic Spray was used daily as a disinfectant agent at the operative sites. The device was activated after 5 days of latency at a rate of 1mm/day for 10 consecutive days. This rate was chosen as it was considered a rapid rate of osteogenesis that results in formation of bone of inferior properties both mechanically and histologically (2, 13). The consolidation period lasted for 25 days starting after the end of the activation period. The total experimental period was 40 days.

3. Regime of Sodium fluoride treatment

Sodium fluoride in drinking water was given twice daily at 12 h interval to study population at two different concentrations. A low dose concentration (10 mg/kg body weight) was specified to group I whereas group I received a high dose (20 mg/kg body weight). The medicated water was held until it was totally consumed by animals. These doses were selected based on literature review of comparable animal species.

4. Methods Evaluation

By the end of experimental course, the animals were slaughtered and the mandible was dissected and hemi-sectioned at the area of the symphy-sismenti. One hemi-mandible was processed for routine histopathological examination and histo-morphometric analysis, the other hemimandible processed for biomechanical testing.

4.1 Histopathological Method

This hemi-mandible was fixed for 1 week in 10% buffered formalin and subsequently decalcified in with 0.5 M Ethylenediamine tetra acetic acid (EDTA) (pH 7.4). The section of mandible containing the distraction zone was trimmed to encompass the area of regenerate bone including at least 2.5 mm of host bone proximal and distal to the regenerate, then sagittally hemisected and processed for routine histopathology by embedding in paraffin. Sagittal sections (6 mm thick) were cut through the full thickness of each specimen and were stained with haemotoxylin and eosin and examined by light microscopy and histomorphometry.

4.2 Histomorphologic quantification

Images were captured using a high-resolution CCD camera (Olympus DP10) attached to light microscope and saved as a digitized 24-bit color TIFF format.Six regions of interest (microscopic fields) were selected for each distraction site; two regions at the proximal part of the regenerate bone adjacent to the old host bone, two regions at the distal regenerate bone and also adjacent to the old host bone and finally two regions in the center of the regenerate bone. Trabecular count, size of individual bone trabeculae, and the total surface area of bone trabeculae in the microscopic field were calculated from H&E stained sections, using image analysis software (Image], 1.37v, USA).

43 Biochemical Properties

The hemi-mandible was mechanically tested using universal testing machine (Lloyd Instruments LR 5 K) at the dental material department, faculty of dentistry, Ain Shams University, Egypt. The specimens were stored frozen in 0.9% sodium chloride at -5°C, and defrosted on the day of testing. The fracture resistance on shear of the distracted bone was measured and the maximum load was calculated using the material testing machine.

4.4 Statistical Analysis

Data were collected and tabulated using Microsoft Excel 2003 and then were statistically analyzed using SPSS version 15. Analysis of variance (ANOVA) was performed and when it yielded a significant result, post-hoc Bonferroni test was used as a pair-wise test. All statistical analyses were performed at the significance level of $P<0.05$.

RESULTS

1. Histopathological Findings

H&E histological examinations of the distracted zones showed similar features in all groups. The middle of the distracted area was found to consist of a fibrous central zone where osteoid tissue is deposited with collagen fibers oriented parallel to the direction of distraction. Ossification appeared to form on either end of the fibrous central zone, resulting in a bridge of immature bone across the distraction gap (figures 2).

2. Histomorphologic Results

The analysis of variance for the parameters used in the current study showed insignificant differences in trabecular count between any of the experimental groups. On the other hand, the fraction area showed a significant difference among these groups, hence Bonferroni post-hoc test was performed as a pair-wise test to analyze these differences and its results showed that the fluorides in low and high dose resulted in significant increase in the area fraction of bone trabeculae compared to the non-fluoride control group (p-value <0.001). However, there was no significant difference between the low and high-dose fluorides regarding the area fraction of bone formed in the distraction zone. Box plots of trabecular area fraction (%) and trabecular count in the 3 studied groups. The number of bone trabeculae formed in the distraction zone was plotted against the area fraction which represents the surface area of bone trabeculae. The plot showed that there was a positive correlation between the surface area and the number of bone trabeculae, however this relation was statistically insignificant.

3. Biomechanical testing

Analysis of variance showed that there was a significant difference between the experimental groups. Post-hoc test showed that fluorides in low dose or high dose significantly increased the fracture resistance of distraction area compared to control group (p-value <0.001). Also in similar a trend to that of area fraction, there was no significant difference between the low doses versus the high dose fluoride groups regarding the fracture resistance of the distraction zone.

DISCUSSION

Fluoride has been used extensively in the treatment of osteoporosis and has been approved by regulatory agencies in many European countries. Based on this finding the current study hypothesized that the beneficial effect of fluorides could be extended to enhance the bone quality and quantity during distraction osteogenesis. In the present study, both concentration of fluorides (10 mg/kg and 20 mg/kg body weight) were found to increase the surface area of bone trabeculae in the regenerate bone but it failed to significantly increase the number of bone trabeculae in the regenerate bone. This unexpected result might be owing to the small sample size, also it is possible that fluorides exert its role by increasing the function rather than the number of osteoblasts implicating that no more osteoblasts are differentiating from the undifferentiated mesenchymal cells. However, the later assumption appears to be unreasonable as the mitogenic action of fluorides in vivo have been recognized in many reports (14,15). Moreover, the mitogenic effect of fluorides in vitro (tissue culture) has been reported. Our results indicated that fluorides improved the mechanical properties of regenerate bone in the distraction zones. This finding might be attributed to the increased amount of newly formed bone trabeculae resulting from the increased surface area of bone trabeculae, or to the improved quality of the newly formed bone resulting from subtle increase in mineralization or collagen formation. Speculative reasons for this improvement include the possibility of increased production for extracellular binding proteins such as osteocalcin, osteopontin and osteonectin.

These extracellular proteins notably, osteopontin is a prominent protein at cement lines in alveolar bone and elsewhere, and here most likely plays a role in cell adhesion and the various extracellular matrix events that integrate newer bone with older bone during the remodeling cycle (15). The beneficial effect of fluorides on distraction osteogenesis found in the current study was corresponding with their positive effect on fracture healing. Similar results were reported by Bialecki who concluded that the use of sodium fluoride led to accelerated osteogenesis including temporary callus formation and mineralization of the new bone. The increased surface area of bone trabeculae noted in the current study might be due to the mitogenic effect of fluoride on osteoblast in vivo and in vitro (14,15).

Another speculative reason for this increase in surface area of bone trabeculae might be the ability of fluorides to inhibit osteoclasts(12,13). In alignment to our findings, several recent studies yield positive outcomes and strongly suggest that the fluoride could be used as therapy in the treatment of osteoporosis9,10,11) In the present study the effect of high dose fluorides was not statistically different from that of low dose fluorides, with consideration of the limited sample size in our study it is possible that the low dose fluorides was fortunately close to the optimum dose. The safety margin of fluoride dose is very narrow and fluorides in high doses have been shown to be toxic to all hard tissue forming cells including osteoblasts (13,14). Fluoride displayed a biphasic pattern of stimulation, this suggests that multiple pathways might be activated. It is possible that low, sub-toxic doses do stimulate proliferation, but at higher doses other pathways responsible for decreasing proliferation or increasing apoptosis might become activated. Characterization of the optimum dose of fluorides to be used in healing enhancement during distraction osteogenesis needs further studies particularly in unfavorable situations such kidney or liver diseases. These studies are crucial to maximize the beneficial effect while minimizing the associated side effects of fluorides.

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