Changes in Inferior Alveolar Nerve Following Mandibular Distraction in Dogs

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INTRODUCTION

Distraction osteogenesis may be regarded as the directionally induced osseous regeneration under the mechano-biological stimulated tensile strain. Ilizarov, a Russian physician, discovered the concept of distraction osteogenesis when he noted a spontaneous osteogenic activity during the course of lengthening an anklyosed knee. Later on, he refined the distraction osteogenesis process by identifying the mechanical and biological guidelines to obtain ideal skeletal regeneration including the latency period, the rate and rhythm of distraction and the consolidation period. Ilizarov stated that DO allowed for a reduction in disability, number of procedures and length of treatment.

Carter et al., [3] investigated the influence of application of mechanical forces, on bone induction process. Their studies aimed to show how the patterns of tissue differentiation, during healing of fracture or distraction osteogenesis, into fibrous tissue, cartilage or bone could be predicted. However, the relation between the physical forces and the process of tissue regeneration remains incompletely understood.

Distraction osteogenesis (DO) is one of the current methods used for bone lengthening and remodeling. It was initially used for the bones of the extremities. Recently there have been several attempts to use this technique in the cranio-facial skeleton for correcting a variety of deformities, jaws discrepancies and congenital malformations and bony defects [7,17].

The effect of osteo-distraction on bone has been reported by many authors [20]. These reports presented contradictory results that might be attributable to small sample size or dissimilar study protocols. In the mean, little is known about the effect of distraction on the adjacent soft tissues and inferior alveolar nerve.

Considering all of these factors, the present study was carried out to evaluate, experimentally, the changes in bone and inferior alveolar nerve after gradual lengthening of the mandible with two different rates and lengths of distraction.

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ABSTRACT

It is well known fact that the mechanical or physical potentialities can influence the biological tissue behavior. In this current study this biological tissue response was investigated through gradual distraction of the mandibular bone. This study was performed on 20 dogs divided to two groups. The first group (group I) included 10 animals subjected to corticotomy at the angle of the mandible then distracted on either side of the corticotomy line by external distractor for 10 mm length using two different rates of distraction. Half of this group (5 dogs) underwent distraction rate of 1.0 mm / day for 10 days, while for the rest of the group the distraction rate was 2.0 mm / day for 5 days. The second group (group II) included the other10 animals subjected to the same procedures undertaken to the animals of the first group but the distraction was performed for 20 mm distance. In each subgroup of the two main groups one animal was subjected to bilateral corticotomy but distracted only at one side. The non-distracted side was taken as a control. Dogs were left for consolidation period of 6 weeks before they were sacrificed. The results indicated that the biomechanical distraction has induced lengthened skeletal repair simulated in the interstitial bone growth at the corticotomy site. In the mean time, the osteogenic activity was denser with the distraction rate of 1.0 mm per day than that with 2.0 mm per day. The Histological examination of distracted part of mandibular bone revealed an area of fibrosseous tissue containing collagen fibers interspersed with fibroblasts and small blood vessels. Many newly formed bone trabeculae composed mainly of immature woven bone were also showed, as well as small foci of bone resorption. Inferior alveolar nerve changes, comprising signs of severe degeneration, marked separation of the perineural sheath, decrease in number of axons and intervening areas of edema were seen in animals subjected for increased length with higher rate of distraction. In conclusion, the results revealed the capability of osteodistraction technique to stimulate osteogenesis, bone remodeling and also the deleterious effect on the inferior alveolar nerve following gradual mandibular osteodistraction in the dog.
Materials and Methods

The current study was performed on 20 skeletally mature and pathogen-free experimental Mongrel dogs with an average weight of 20 kg (18 kg – 26 kg) and age ranged from 10-14 months. The animals were divided into two groups. The first group (group I); included 10 animals subjected to corticotomy at the angle of the mandible then distracted on either side of the corticotomy line by external distractor for 10 mm length. This distance was achieved twice daily and with two different rates of distraction. Half (five dogs) of this group (group I-a) underwent distraction rate of 1.0 mm/day for 10 days, while for the rest of the group (group I-b) the distraction rate was 2.0 mm/day for five days. The second group (group II) included the other 10 animals subjected to the same procedures undertaken for the animals of the first group but for 20 mm distraction length. The group was divided into two groups. Group (II-a) included five dogs and underwent distraction rate of 1.0 mm/day for 20 days, while the rest of the group involved the remaining five animals (group II-b) and underwent distraction rate of 2.0 mm/day for 10 days. In each subgroup of the two main groups one animal was subjected to bilateral corticotomy but distracted only at one side. The non-distracted side was taken as a control. Dogs were left for consolidation period of 6 weeks before they were sacrificed. During this period the devices were stabilized with acrylic resin.

The surgical procedures of the mandibular corticotomy were established under general anesthesia using 1mg/kg body weight ketalar* in combination with 0.1 mg/kg body weight 2% Rumpun** injected intravenously. Through a submandibular approach, skin incision below and parallel to the inferior border of the mandible was made then reflecting the skin, subcutaneous tissues and masseter muscle. The periostium was incised and carefully reflected to preserve its integrity. Two bicortical drill holes (using 2.7 mm drill) were done 1 cm on either side of the planned corticotomy line at the ramus body junction of the mandible defined by blue marker, then two self tapping stainless steel pins(5cm long & 3.5 mm diameter) were inserted into the holes to engage the lingual cortices.

The corticotomy line was made in the buccal and lingual cortices using both round and fissure surgical burs mounted on straight handpiece with low speed (5000 rpm) under water irrigation (fig.1). Before complete separation of the bony segments and cutting of the inferior border of the mandible (using osteotome), the prefabricated external unidirectional distractor was fixed to the mandible through the pins in a perpendicular plane to the direction of corticotomy line (fig.2). The soft tissues were then sutured in layers. Routine postoperative regimen was followed as proposed.

After latency period of 7 days the mandibular distraction was started by activating the external distractors at the corresponding rate and time designed for each group. After the end of the distraction phase the mandible was subjected for a fixation period of 6 weeks during which the distractors were stabilized and not activated. This period was intended to allow adequate time for the osseous consolidation of the distracted mandibular segments to maintain a structural integrity.

* Ketalar (ketamine hydrochloride) Park Davis Company, USA.
** Rumpun Bayer Company, Germany.

Continuous monitoring for the tight fixation of the distractors, sepsis or loosening of pins and assurance that no movements at the distracted sites were constantly done throughout the work. After the fixation period, the animals were killed with overdose of sodium pentobarbiton administered intravenously. The distractors were removed, the mandible was dissected out and the mandibular segment between the bony holes was cut with a safety margin. The distracted bone segment was fixed in 10% formol saline for 72 hours and decalcified in 20% sodium citrate and 5% formic acid. The decalcified tissue specimens were micro technically processed to obtain \( \mu \)m thick sections parcelling the outer surface of the mandible. The sections were evaluated for routine decalcified morphologic properties under light microscopy applying Harris’s hematoxylin and eosin and silver staining.

Results:

The current study was conducted on 20 dogs. All animals underwent unilateral mandibular body distraction. The distraction sites were approached via submandibular incisions. The clinical postoperative follow up was uneventful for most of the dogs. Most animals tolerated the surgical procedures without complications. No signs of wound infection or evidences of soft tissue breakdown were observed in any of the dogs throughout the study.

Regular postoperative follow up was done to check the fixation of the distractors that proved rigid and stable and allowed proper and regular distraction of the bony segments. At the end of the consolidation period, the distractors were removed under Rumpun sedation. Most animals developed stable and firm regenerate bone. Two dogs suffered from non-union of the distracted bone after surgery. These dogs were in group (II-b) that underwent distraction for 20 mm with a rate of 2.0 mm/day. Lengthening of mandibular body was achieved in all dogs with deviation to the non-distracted side. Throughout the follow up period, the animals were apparently in good general condition and non of them died until the sacrificing time.
Histological examination of the distracted bone segments of all dogs used in this study revealed nearly the same findings, as seen clearly in all tissue sections stained with hematoxylin and eosin. An area of fibro-osseous tissue formation was revealed within the distraction gap. It consisted of a background of fibro-adipose tissue comprising collagen fibers arranged individually or grouped into bundles, a variable numbers of fibroblasts and many small congested blood vessels in between the bone trabeculae. (Fig.3). Within the area of fibro-adipose tissue, many small spicules of newly formed bone trabeculae were seen traversing the distracted part of the mandible. Most of the newly formed bone was formed of immature woven bone in type that appeared branched and anastomosed together in form of a network which ultimately developed haversian system (Fig.4). Some newly formed bone spicules, arising from the osteotomized bone edges, were bordered with a continuous layer of osteoblasts that indicating the healing activity. Moreover, bone density seemed to be greatest along the edges of the original bone, while less bone density at the center of the distraction osteogenesis gap was seen (Fig. 5).

The most interesting finding was the close relation between the newly formed bone spicules with the distraction. Most of the old bone spicules were fractured and the surrounding blood vessel were seen ruptured with extra-vasation of blood. In other occasion, the bone spicules were closely attached to the walls of blood vessels (Fig.6). Whether this might represent an accidental event or have an actual relation, no conclusion can be proposed. However, the vasculo-osseous relation represented common feature. However, the specimens that underwent high distraction rate (2.0 mm/day) revealed continuous and progressive bone remodeling including bone resorption or even degradation. The bone degradation was the most common sign in which the bone trabeculae disappeared leaving spaces containing some debris within the surrounding fibrous connective tissue with no osteoclasts. Surface bone resorption was also noted as evidenced with the presence of osteoclasts (Fig. 7).

Histological evaluation of the distracted portion of the inferior alveolar nerve of the experimental animals, as observed obviously in tissue sections stained with hematoxylin-eosin and silver, revealed variable changes in the nervous tissue. Little conspicuous histological alteration was noticed in cases with distraction rate of 1.0 mm/day and shorter length of distraction. Mild to moderate degeneration of the nerve fibers was also seen in some cases underwent greater distraction lengthening with the same rate of distraction 1.0 mm/day (Fig. 9&10). In the mean time signs of active degeneration comprising marked separation of the perineural sheath, absence of many nerve fibers with intervening edematous areas and decrease in number of axons were seen in cases underwent greater distraction length with high distraction rate (Fig. 8). Disappearance of neurofibrilis and a significant increase of fibrous density were also noted. Moreover, the peripheral nerve fibers and their terminal branches showed marked degenerative changes (Fig. 11&12).

![Fig. 1: Intra-operative photograph showing the corticotomy line with the two pins in place. Note retraction of periostium and overlying soft tissues.](image-url)
Fig. 2: Photograph showing soft tissues closure and fixation of the distractor.

Fig. 3: Expended area, showing a background of fibro-adipose tissue containing collagen fibers, fibroblasts and blood vessels congested with blood in between the bone trabeculae. (H&E Stain, X 63).

Fig. 4: Other area of expanded part, showing active distraction osteogenesis near the surface of the mandible. Bone trabeculae appeared branched and anastomosed together to form network in preparation of haversian system development. (H&E Stain, X 63).
Fig. 5: Center of the distracted area, showing newly formed non lamellar bone trabeculae bordered with continuous layer of osteoblasts and less bone density. (H&E Stain, X 100).

Fig. 6: Bone trabeculae are seen closely related to wall of blood vessels. (H&E Stain, X 100).

Fig. 7: Mid portion of the distracted area, showing many small woven bone spicules in a background of fibrous tissue. Woven bone resorption through the activity of several osteoclasts was also noted. (H&E Stain, X 100).
Fig. 8: Inferior alveolar nerve of experimental animals, showing separation of perineural sheath, absence of many nerve fibers and edematous areas. (H&E Stain, X 100).

Fig. 9: Inferior alveolar nerve, showing mild degeneration of nerve fibers. (Silver Stain 100).

Fig. 10: Inferior alveolar nerve, showing moderate degeneration of nerve fibers. (Silver Stain, X 100).
Discussion:

It is well known that mechanical or physical potentialities can influence the biological tissue behavior [9]. However, the interaction between the mechanical and biological factors during the tissue differentiation is not completely understood (Block and Hoffman, 1995). Also, the modality by which the local strain environment can affect tissue repair is not clear [6].

Complete immobilization of the osteotomised segments was attained throughout the study. This was of a paramount importance to avoid any passive effect on osteoinduction process. Bone movement perhaps disturbs the local vascular regeneration and diminishes the oxygen tension that affects the repair process [1]. Optimal controlled distraction with good fixation of bony segments might be regarded as osteoinductive rather than osteoinhibitor.

In the current work the neurovascular bundle was preserved to enhance tissue regeneration. It was emphasized that inter-medullary blood supply could serve as a potent biologic stimulus for bone healing. This coincided the findings of Yasui et al., [23] who reported that inter-medullary blood circulation was essential to allow bone regeneration after distraction. This was in contrast to Costantino et al., [5] who completely transected the neurovascular bundle. They reported that preservation of inter-medullary blood supply was not necessary for success.

In their study, Monasterio et al., [18] left the inner cortex intact during surgery and await fracture during distraction. In contrast to this concept complete separation of the bony segments at the time of osteotomy was achieved in the current study. This was thought to eliminate the possibility of complications associated with incomplete segment mobilization as premature consolidation or the need for second surgical procedure. This technique was recommended by Cornelius Klein [4] who added that complete separation of the segment would place more control in the hands of the surgeon, which is...
more predictable than waiting a time in which the bone yields to distraction forces.

The extraordinary rich vasculature at the center of moderately distracted area (1.0 mm/day) compared with the regular vascularization in the corresponding non-distracted control area indicated that the distraction enhanced the process of neovascularization. Such vasculature was necessary for the success of skeletal repair [11]. In the current study corticotomy, rather than the osteotomy, was done to preserve the intramedullary vasculature and optimize bone regeneration.

Many factors affect the integrity of tissue repair during distraction process. The latency period, the rigidity of fixation, the rate and rhythm of distraction and the degree of tissue damage are some of these factors. Both of the rates and the rhythms of the applied tensile strain were thought to be significant in influencing the amount and type of the tissue repair after distraction. This was evidenced in the present study as the moderate intensity of tensile strain (1.0 mm/day) was found to stimulate the tissue differentiation and intra-membranous ossification whereas the high tensile strain (2.0 mm/day) was found to produce deleterious effects on the distracted tissues. Therefore, the controlled mechanical stimulation has been documented as enhancing factor in tissue healing. These findings coincided the results of Makarov et al., [14] who stated although soft tissue adaptation requires a slower distraction rate than the bone, premature consolidation of the bone regenerate may occur. They recommended distraction rate of 0.75 -1 mm per day to be optimal to balance bone and soft tissue regeneration.

Histological findings of the mandibles distracted with a rate of 1.0 mm / day showed a background of fibroadipose tissue containing many small bone trabeculae traversing the distracted part, as well as some newly formed bone spicules arising from the osteotomized bone edges. This coincided the findings of Sato et al., [20]. This moderate distraction rate together with the preservation of intact periostium over the osteotomy site appeared to achieve early new bone and callus formation not only at the center of the expanded area of the mandible but also continue to be progressively increased as far as the unexpanded bone ends. This explanation was more supported by Mchrara et al.,[16].

Rapid distraction was originally regarded as a method of shortening the treatment time. In the cases that underwent distraction rate of 2.0 mm/day, histological evaluation revealed progressive bone remodeling including bone resorption and degradation. Bone trabeculae disappeared leaving spaces surrounded by fibrous connective tissue containing some persisting remnats. Many areas showed bone resorption as evidenced with the presence of osteoclasts (fig.7). In the current study two dogs suffered from non-union after distraction. These dogs underwent distraction rate of 2.0 mm/day and distraction length of 20 mm. These two cases of non-union might be attributed to the high rate and the greater length of distraction that interfered with proper formation and ossification of bone. These results coincided the findings reported by Stewart et al., [22] who stated that rapid distraction resulted in a greater incidence of non-union during mandibular osteogenesis.

In the present study examination of inferior alveolar nerve revealed little histological changes in the cases distracted for 10 mm with a rate of 1.0 /day. This coincided the findings of Block and Hoffman [2], who reported no conspicuous alteration in nerve fibers when 5-10 mm of mandibular lengthening was accomplished. This was also more supported by Makarov et al., [14] who concluded that distraction osteogenesis with 10 mm lengthening appeared to produce minimal deleterious effect on IAN function assuming no acute nerve injury occurred during surgery. In the mean time, the results of the current work agreed Karp et al., [12], who reported absence of myelinated nerve fibers in the IAN after 20 mm of mandibular bone lengthening despite attempts for preservation of the neurovascular bundle during surgery.

Histological evaluation of inferior alveolar nerve (IAN) specimens distracted with high rate (2.0 mm / day) revealed marked separation of the perineural sheath, decrease in number of axons and signs of active degeneration of nerve fibers (fig.11,12). These findings came in accordance with the results reported by Skoulis et al., [21] and Polo et al., [19]. The deleterious effects on IAN were most likely due to a combination of a mild mechanical deformation, compression of the IAN against the wall of the canal, and impairment of venous blood flow of the nervous tissue. This was more supported by Jing et al., [8] who also added that distraction rate of 1.0mm/day appeared to be safe and tolerable, but rapid distraction (2.0 mm/day) might cause serious degeneration of the nerve. Moreover, the postsurgical neurogenic changes found in the current study were apparently more frequent especially with advancements of greater lengths (20mm).

Distraction osteogenesis presents a unique form of clinical tissue engineering. The clinician is able to guide the formation of new tissues with a controlled gradual distraction. This occurs without the application of any growth factors or other pharmacologic agents. Careful a traumatic surgical procedure, preservation of periostium, the neurovascular bundle and inter-medullary blood supply and proper fixation of the osteotomised segments are of paramount importance in distraction process. Moderate distraction rate (1.0 mm/day) and reasonable distraction length (15 mm) are also important factors to achieve successful results in distraction osteogenesis.

The present study revealed that both bone and IAN responded differently to various rates and
lengths of distraction. However the mechanism of bone and soft tissue adaptation to gradual distraction is still not completely understood. Also, considerable work is still required to clarify how histological changes affect nerve function clinically.

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