Int. J. Oral Maxillofac. Surg. 2015; 44: 404–411
http://dx.doi.org/10.1016/j.ijom.2014.10.002, available online at http://www.sciencedirect.com

International Journal of Oral & Maxillofacial Surgery

Research Paper
Bone Healing

The effect of human amniotic fluid on mandibular distraction osteogenesis

S. M. Gokce[1], U. Karacayli[2], R. Nalcaci[3], M. C. Avunduk[4], M. Özgöçmen[5], E. Karasahin[6], H. S. Gokce[7]

[1]Dental Sciences Centre, Department of Orthodontics, Gulhane Military Medical Academy, Etlik, Ankara, Turkey; [2]Department of Maxillofacial Surgery, Gulhane Military Medical Academy, Etlik, Ankara, Turkey; [3]Department of Orthodontics, Suleyman Demirel University, Isparta, Turkey; [4]Department of Pathology, Meram Medical University, Isparta, Turkey; [5]Department of Histology, Suleyman Demirel University, Isparta, Turkey; [6]Department of Gynaecology and Obstetrics, Gulhane Military Medical Academy, Etlik, Ankara, Turkey; [7]Department of Prosthetics, Medipol University, Mega Hospitals Complex, TEM Autoban Goztepe exit Bagcilar, Istanbul 34214, Turkey

S.M. Gokce, U. Karacayli, R. Nalcaci, M.C. Avunduk, M. Özgöçmen, E. Karasahin, H.S. Gokce: The effect of human amniotic fluid on mandibular distraction osteogenesis. Int. J. Oral Maxillofac. Surg. 2015; 44: 404–411.

Abstract. The aim of this study was to evaluate the effects of local administration of human amniotic fluid (HAF) on newly formed bone obtained by mandibular distraction osteogenesis (DO) with histomorphometry. A unilateral mandibular osteotomy at the left corpus was performed in 32 adult male rabbits. After a 5-day latency period, the left mandibles were lengthened by mandibular DO over 5 days, at a rate of 1 mm/day, via a custom-made distractor. After the distraction, the rabbits were divided randomly into four groups: 0.3 ml HAF was injected into the distraction gap followed by 21 (group 1) or 45 (group 2) days of consolidation; or 0.3 ml normal saline (NS) was administered followed by 21 (group 3) or 45 (group 4) days of consolidation. Mandibles were removed at the end of the consolidation period and investigated histomorphometrically. The newly formed bone area (NFBA) and number of fibroblasts increased significantly in the HAF groups compared to the NS groups (NFBA: group 1 vs. group 3, $P < 0.05$; group 2 vs. group 4, $P < 0.01$; fibroblasts: group 1 vs. group 3, and group 2 vs. group 4, $P < 0.05$), and also in both 45-day consolidation groups compared to the 21-day consolidation groups (NFBA: group 1 vs. group 2, and group 3 vs. group 4, $P < 0.001$; fibroblasts: group 1 vs. group 2, and group 3 vs. group 4, $P < 0.01$). Additionally, the numbers of osteoblasts and capillaries were increased significantly at 45 days of consolidation compared to 21 days in both the HAF and NS groups (osteoblasts: group 1 vs. group 2, $P < 0.01$; group 3 vs. group 4, $P < 0.05$; capillaries: group 1 vs. group 2, and group 3 vs. group 4, $P < 0.01$). Histomorphometric analysis demonstrated that local HAF administration effectively accelerated bone formation. Thus, a HAF injection procedure could improve new bone formation around the bone in maxillofacial operations such as DO.

Key words: human amniotic fluid; distraction osteogenesis; histomorphometry; bone.

Accepted for publication 3 October 2014
Available online 15 November 2014

Distraction osteogenesis (DO), used widely in the treatment of oral and maxillofacial deformities or deficiencies, is a biological procedure that produces new bone formation via gradually separated bone segments, using an external lengthener.[1–3] Traditional surgical techniques for skeletal expansion include osteotomies, acute movements of variable magnitude, and the necessity for bone grafts. Problems include donor site morbidity, unpredictable graft resorption, and the risk of relapse because of soft tissue resistance to large skeletal movements.[4] Many of these limitations can be avoided with the use of DO to lengthen or expand the skeleton.[3,4] The benefits of DO include the minimally invasive nature of the procedure, the ability to achieve movements of great magnitude without the need for a bone graft, and the elimination of donor site morbidity.[4] The main advantage of the technique is that the new bone forms together with elongation of the surrounding soft tissue envelope.[1] In addition, concurrent soft tissue histogenesis may decrease the relapse.[5,6]

Although this technique is used to treat several abnormalities of hard and soft tissues, there are some drawbacks, particularly related to long-term consolidation periods.[1] To accelerate the maturation of the regenerated bone, numerous approaches including growth factors,[7] calcitonin,[8] calcium sulphate,[9] bisphosphonates,[1,2] and electronic[10] and ultrasonic[11] stimulation have been researched.

Several growth-promoting factors have been identified after the manifestation of bone defects including those caused by injuries, fractures, and DO; these include platelet-derived growth factor, transforming growth factor beta (TGF-β), fibroblast growth factor (FGF), interleukin (IL)-1, and IL-6. FGF has angiogenic properties and mitogenic activity on the osteoblast lineage.[12] A rich content of growth and trophic factors such as epidermal growth factor (EGF), FGF, and insulin-like growth factors I and II (IGF-I and IGF-II), which are critical for development, have been identified in human amniotic fluid (HAF).[12–15] Additionally, hyaluronic acid (HA), hyaluronic acid stimulating activator (HASA), chondroitin-4- and -6-sulphate, dermatan sulphate, and heparan sulphate have been identified in HAF.[12,16]

HAF, generally obtained by amniocentesis during the second trimester of gestation, contains high molecular weight HA and HASA in high concentrations.[14–17] HA has been shown to increase osteoblastic bone formation in vitro, through increased mesenchymal cell differentiation and migration.[15,17] Also, HASA has been shown to stimulate and increase the production of endogenous HA. Thus, HAF may increase both endogenous and exogenous HA around the region of application.[17]

Several studies have investigated the effect of HAF on cell differentiation. HAF has been reported to enhance new cartilage, bone formation, and nerve and tendon healing.[12–15,17–19] However, new bone regeneration in the distraction gap after DO of the mandible has not been documented, and there are no data in the literature regarding the acceleration of bone regeneration in the distraction gap with the use of HAF. We hypothesized that HAF would have a positive stimulating effect on bone formation after DO. Hence, the aim of this study was to investigate the effects of HAF, collected between weeks 16 and 24 of gestation, on the acceleration of new bone formation in animal subjects undergoing mandibular DO.

Materials and methods

All procedures were performed in the experimental animal breeding and research centre of the military medical academy. Approval was obtained from the institutional ethics committee and local clinical research ethics committee for the animal research and use of HAF.

Subjects and surgery

The study subjects were 32 adult male New Zealand White rabbits of the same age with an average weight of 2.9 kg (range 2.2–3.5 kg). They had free access to a standard pellet diet and tap water and were adapted to a 12:12-h light–dark cycle in separate cages.

All animals were operated on under general anaesthesia. The rabbits were anaesthetized with a combination of xylazine hydrochloride (5 mg/kg) (Alfazyne 2%; Ege Vet, Izmir, Turkey) and ketamine hydrochloride (50 mg/kg) (Alfamine 10%; Ege Vet, Izmir, Turkey) before the application of distractors. Isoflurane inhalation anaesthetic (10 mg/kg) was used before the various procedures.

After sterilization of all surgical equipment, the left mandible was shaved and disinfected with iodine. After surgical preparation of the experimental side of the mandible, a 2–2.5-cm long submandibular skin incision was made. The subcutaneous tissues were exposed by careful dissection down to the periosteum and the bone was exposed with a periosteal incision. Using a reciprocating saw, a vertical corticotomy line was outlined between the premolars and extended to the inferior mandibular border under saline irrigation. The extraoral bone-borne distraction device used consisted of a 7-mm hyrax expansion screw (Dentaurum GmbH & Co., Ispringen, Germany) with three holes; the retention legs were bent for bone attachment. The distractor device was fixed with two posterior (7 mm) and one anterior (9 mm) titanium screws. The bone cut was completed using a thin osteotome through the vertical corticotomy line, and mobilization of the bone fragments was achieved. Activation of the distraction device was tested. The gap between the bone fragments was narrowed by reverse-directed activation of the distractor. The periosteal flaps were repositioned and closed with 4–0 Vicryl sutures (Fig. 1).

After closure of the mandible, the rabbit was awoken from general anaesthesia and allowed to recover. An Elizabethan collar was placed around the rabbit's neck to protect the device from dislodgement. A subcutaneous bolus of 30 ml normal saline (NS) was given every 8 h during the first 24–48 h depending on the rabbit's water intake. After the rabbit had recovered fully as per the facility criteria, it was

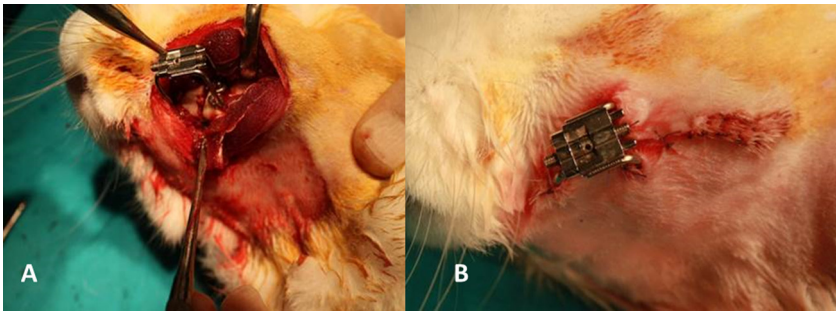

Fig. 1. (A) Placement of the distraction device with three parallel fixation pins, which are perpendicular to the lateral surface of the mandible. (B) At the end of the surgery; unilateral placement of the distraction device.