Distraction Osteogenesis of the Palate: An Experimental Model

John M. Menezes, MD, Ross L. Stutman, MD, Patrick S. Murphy, MD, Linda L. Stephenson, BS, MT(ASCP), and William A. Zamboni, MD, FACS

Abstract: Although most cleft palates can be closed with conventional mucoperiosteal flap techniques, the occasional wide cleft or difficult fistula has few options for an early 1-stage reconstruction. Distraction osteogenesis (DO) has the potential to close the palate with both hard and soft tissues as well as mitigating the possibility of future oronasal fistula. A right unilateral 5-mm cleft was surgically created in 15 New Zealand white rabbits. In group 1 (N = 5), no further repair was performed (NR); in group 2 (N = 5), mucoperiosteal flaps were used to close the cleft for a soft-tissue-only repair (STR); in group 3 (N = 5), a unilateral osteotomy in the palate on the noncleft side allowed distraction of the palatal shelf across the cleft until closed (DO). Clinical examination, Micro-computed tomography bone density, direct cephalometry, and histology were evaluated at 8 weeks after the completion of distraction. Bone mineral density (BMD; mg/mL) data were obtained from micro–computed tomography scans of both the cleft and noncleft sides for each rabbit, and a ratio was obtained [(BMDc/BMNdnc) × 100]; NR = 1.38, STR = 44.27, DO = 88.36, P = 0.007. Facial measurements revealed no growth disturbances as a result of DO. Histologic evaluation revealed increased organization of new bone in DO group compared with NR and STR. Clinically, DO group rabbits did not show any increase in feeding disturbances, infection, or wound healing. The success of membranous facial bone distraction has been applied to a new model for palatal repair with the potential to ameliorate the problems associated with soft-tissue-only repair.

Key Words: Distraction osteogenesis, cleft palate, rabbit, oronasal fistula

The purpose of surgical closure of both cleft lip and palate is to achieve a normal appearance and normal speech. Bernard von Langenbeck (1810–1887) was the first to develop a soft tissue technique of palatal closure that involved a subperiosteal dissection and the movement of mucoperiosteal flaps.1 Although in most cases, a conventional palatoplasty is adequate, there are a certain small percentage of cases in which the cleft defect is wider than repair by soft tissue flaps, tethered by the greater palatine artery will allow. In addition, fistula, which has been reported to occur at a low but steady rate in up to 23%, will remain an issue in any repair that does not also address the bony defect. Anterior fistulas, in particular, can be problematic and require measures such as tongue flap for reconstruction and have a relatively high failure rate after attempted repair, recurring in up to 37%.2 Anterior fistulas can be particularly problematic because the tissue adjacent to the alveolus is limited and contrated palatal tissues are difficult to advance anteriorly. In the 20th century, the subperiosteal repair was criticized by Veau as resulting in a short, immobile palate secondary to scarring.3 During the subsequent decades, many authors have reported retardation of the growth of midface and maxilla after closure of palatal clefts4; this is confirmed in studies comparing operated and nonoperated patients as well.5 Overall, facial growth potential in patients with cleft lip and palate is generally considered normal. In other words, the growth restrictions found in patients with cleft lip and palate is a localized phenomenon and not a craniofacial anomaly due to the fact that a normal growth potential has been observed in all craniofacial regions, except where the growth had been directly influenced by surgical intervention.6 Other studies support the notion that surgically created scar is causal to the midface growth deficiencies seen in children with cleft palate. Surgical correction in 2 stages instead of 1, which introduced more scar, results in greater growth restriction.6 Therefore, wide clefts that may require delayed or staged repair could potentially benefit by early repair with a horizontal distracter, as would an anterior fistula with an anteroposterior distractor.

Distraction osteogenesis (DO) was developed primarily by Gavril Ilizarov in the 1950s and was used to lengthen the long bones of the extremities (enchondral bone). More recently, the technique has been successfully applied to membranous bones of the craniofacial skeleton by pioneers such as McCarthy et al7 primarily in the mandible. After these successes, the technique has been expanded to other bones of the face including the maxilla at the Le Fort I and III levels and even in cranial bone as part of monobloc distraction.8–11 To date, distraction has not been a technique applied as a primary method of human cleft palate repair. The opposite or rapid palatal expansion using a maxillary osteotomy and a palatal expander to correct a constricted arch has been used, but closure of a cleft palate has not.12 Several experimental animal models have demonstrated the feasibility of posterior palatal lengthening using DO.13,14 In a few cases of human submucous cleft palate, in which
the hard palate is intact and the soft palate is deficient, DO has been used to lengthen the palate.\textsuperscript{15} Distraction osteogenesis across a palatal osteotomy is a modality that has been proven in the membranous bone of the craniofacial skeleton and that potentially can close both the bony and soft tissue defects of patients with cleft palate.

**MATERIALS AND METHODS**

This protocol was approved by the Institutional Animal Care and Use Committee of the University of Nevada, Las Vegas, before its initiation. Fifteen male New Zealand white (NZW) rabbits (Western Oregon Rabbit Company, Philomath, OR) weighing between 2.0 and 2.5 kg were used in the study. Upon receipt of all animals, the staff of the Department of Comparative Medicine, University of Nevada, Las Vegas, examined all animals for any preexisting abnormalities or defects. Once accepted, the rabbits were housed individually in cages in humidity- and temperature-controlled rooms. The animals were given standard rabbit food and water at libitum. At ages 10 to 12 weeks, the animals were randomly divided into 3 groups of 5 rabbits each: group 1 (no repair = NR), palatal cleft creation without further intervention; group 2 (soft tissue repair = STR), palatal cleft creation with mucoperiosteal flap repair; and group 3 (DO), palatal cleft creation repair with distraction device.

**Surgical Procedure**

The animals were anesthetized with an intramuscular (IM) injection of 35 mg/kg of ketamine and 5 mg/kg of xylazine (Am Tech, St Joseph, MO). After sufficient anesthesia, each rabbit received 1 dose of benzathine and procaine penicillin G (Bicillin) 2,500 IU/kg IM (King Pharmaceuticals Inc, Bristol, TN). Once asleep, the rabbit’s palate was prepared with betadine (10% povidone iodine) and 70% ethanol for antisepsis and draped with a sterile towel to prevent contamination. After induction of anesthetic and preparation of the surgical site, a unilateral palatal cleft was created in a manner similar to that described by Bardach et al.\textsuperscript{16} A strip of mucoperiosteum was directly excised after injection of a local anesthetic (1% lidocaine hydrochloride with epinephrine 1:100,000; Abbot Labs, North Chicago, IL). Sufficient mucosa was maintained to allow repair of mucosa over the border of the created bony cleft so as not to leave exposed bone. A 5-mm cleft was then created in the palatal bone with a surgical burr followed by repair of the nasal to the oral mucosa at the cleft margin with a 5-0 Vicryl suture (Ethicon Inc, Somerville, NJ) (Fig. 1).

For the group receiving soft tissue repair (group 2), lateral palatal incisions were made 0.5 mm lingual to the alveolus and mucoperiosteal flaps developed preserving the neurovascular pedicles to the flaps. The flaps were then repaired in the midline with additional sutures placed at the nasal mucosa to eliminate potential dead space (Fig. 2). Group 3 did not have a soft tissue repair. Here a lateral osteotomy was made on the noncleft side with perforations created with a drill bit and completed with an osteotome. The distraction mechanism, a modification of the Micro Zurich infant mandibular distraction device (KLS Martin, Jacksonville, FL) was placed across the cleft with titanium screws securing the device bilaterally (Figs. 3 and 4).

Postoperatively, all rabbits were observed until fully awake. One dose of buprenorphine 0.01 mg/kg IM (Reckitt Benckiser Pharmaceuticals, Inc, Richmond, VA) was given intraoperatively and then every 12 hours for 2 days for analgesia. All animals were observed for signs of pain and distress (such as agitation, inability to ambulate, decreased food and water intake) on a daily basis for 3 days and then for 3 days every week. Food and water consumption was monitored throughout the study. All animals were weighed preoperatively, at 1-week intervals throughout the study, and at the time of euthanizing.

No additional manipulation was performed after the initial operation for groups 1 and 2. For group 3, after a 5-day latency period, distraction was started with 0.3-mm turns to the device twice a day for a total of 10 days to close the 5-mm defect. After the

FIGURE 1. Group 1, no repair group. Preoperative photograph of rabbit palate (A) and 5-mm unilateral cleft created (B).

completion of distraction, a bone consolidation period of 8 weeks was allowed to occur before administration of euthanasia and examination of the tissues. At week 9, all rabbits were euthanized by lethal doses of intravenous pentobarbital.

Tissue Harvesting

All rabbits were immediately decapitated, and all soft tissue to the level of periosteum was removed, with the exception of the palatal mucosa. The distraction device was removed from all animals in which it was placed. Lastly, the mandible was disarticulated and discarded. All skulls were then placed in formalin for preservation.

Skull Measurements

Direct cephalometry was performed on each of the rabbit skulls to evaluate any changes in facial growth between the 3 treatment groups. The following landmarks were identified and marked on the skull surface.

- M: medial margin of the alveolus of the right first molar
- N: medial margin of the alveolus of the left first molar
- O: medial margin of the alveolus of the right last molar
- P: medial margin of the alveolus of the left last molar
- M-N (anterior maxillary width)—length between the medial margins of the alveoli of the first molars
- O-P (posterior maxillary width)—length between the medial margins of the alveoli of the last molars
- Y-Y’ (posterior facial width)—length between frontal bone lateral margins in front of posterior supraborital process
- Z-Z’ (posterior facial width)—length between posterior point of zygomatic-squamosal suture

Micro-Computed Tomography

Each rabbit skull was sent to San Antonio Children’s Cancer Research Institute for micro-computed tomography (CT) evaluation. Micro-CT measurements of bone mineral density (BMD) and bone mineral content (BMC) were performed of the region of interest using GE Microview software (General Electric Healthcare, Waukesha, WI).

FIGURE 3. Distraction device (KLS Martin).

FIGURE 4. Group 3, DO. Intraoperative view of distraction device in place before initiation of distraction.

FIGURE 5. Direct cephalometry technique. A, Ventral view of landmarks identified and measured. M-N was used to measure anterior maxillary width; O-P, posterior maxillary width. B, Dorsal view of landmarks Y-Y’ and Z-Z’ posterior facial width.

FIGURE 6. Changes in body weight of each treatment group from beginning (date of cleft creation ± soft tissue repair or placement of distraction device) to final (9 weeks after initial cleft creation). Note: error bars represent SEM.
Each palate was also measured at the opposite noncleft portion of hard palate. This was performed to allow each skull to serve as its own control, as well as give absolute quantitative results.

**Histology**

All skulls were sent in formalin to Quest Diagnostics (Las Vegas, NV). Each palate was sectioned per Quest Diagnostics and stained with hematoxylin and eosin (H&E). All histologic evaluation was performed by a single pathologist blinded to the treatment group of each skull.

**Statistical Analysis**

Group differences across the measured parameters were statistically analyzed using analysis of variance. All calculations were performed using Stat Pak 4.1 (Portland, OR). A $P < 0.05$ was considered significant.

**RESULTS**

**Body Weight**

There were no significant differences in body weight between the 3 treatment groups at the time of operation: group 1 (NR), mean weight of 2.53 kg (SEM, 0.18); group 2 (STR), mean weight of 2.54 kg (SEM, 0.03); and group 3 (DO), mean weight of 2.48 kg (SEM, 0.06). All animals consistently gained weight throughout each week of the 9-week study. At 9 weeks, there was no significant difference in overall weight gained between groups (Fig. 6).

**Clinical Impressions**

There were no complications of infection, wound healing, or mechanical failure of the distraction device. All animals were able to eat and drink without obvious difficulty. Clinical observation at 9 weeks of palatal closure revealed a more organized soft tissue arrangement overlying the cleft portion of the palate in the DO group compared with the STR and NR groups. All groups seemed to have at least some bone formation at the cleft site (Figs. 7–9).

**Skull Measurements**

Direct cephalometry was performed to evaluate any changes in skull growth throughout the treatment groups. Anterior maxillary width was measured by anterior maxillary width. There was no significant difference between groups because the mean lengths in millimeters for the NR, STR, and DO groups were 11.9 (SEM, 0.17), 11.4 (SEM, 0.4), and 11.3 (SEM, 0.44), respectively. Posterior maxillary width was measured by posterior maxillary width.
There was also no significant difference between treatment groups. The mean posterior maxillary width for the NR was 13.0 (SEM, 0.63), STR was 13.80 (SEM, 0.73), and DO was 13.80 (SEM, 0.41).

Posterior facial width was evaluated using $Y$ and $Z$ measurements for the NR, STR, and DO in millimeters were 14.4 (SEM, 2.16), 14.0 (SEM, 1.79), and 17.1 (SEM, 2.42). The $Z$-measurements were 45.5 mm (SEM, 1.45) for the NR group, 44.6 mm (SEM, 1.03) for the STR group, and 42.7 mm (SEM, 1.43) for the DO group.

Overall, there were no significant differences between the treatment groups with regard to facial growth (Fig. 10).

**Micro-CT**

Micro-CT was used to evaluate both BMC in milligrams and BMD in milligrams per milliliter. Each animal also served as its own control by evaluating the noncleft side of the palate (BMCnc and BMDnc) to the cleft side (BMCc and BMDc).

Bone mineral content of the cleft side was significantly reduced in the NR group compared with both the STR and DO groups ($P = 0.0064$ and $0.0057$, respectively). Interestingly, although there was a slight increase in BMC of the DO compared with the STR, this did not meet statistical significance. There was also no significant differences in BMCnc between the NR and STR groups; however, there was a significant difference between the STR and DO groups ($P = 0.0093$). This likely was due to the decreased density of the osteotomy site in the DO group (Fig. 11).

Bone mineral density of the cleft side was also significantly reduced in the NR group compared with both the STR and DO groups ($P = 0.0078$ and 0.0087, respectively). However, there was no significant difference in BMDc between STR and DO. As with BMCnc, BMDnc revealed similar findings, with no significant differences between the NR and STR groups or NR and DO groups. However, a significant difference between the STR and DO groups ($P = 0.0042$) did exist, again likely due to the incomplete ossification of the osteotomy site (Fig. 12).

Each group was also compared as a ratio of BMCc/BMCnc and BMDc/BMDnc to better evaluate these measures using each group as its own control. The BMCc/BMCnc ratios for the NR, STR, and DO groups were 3.01%, 44%, and 89.9%, respectively. This did prove statistically significant for all groups; NR compared with STR, $P = 0.0099$; NR compared with DO, $P < 0.0001$; and STR compared with DO, $P = 0.0035$ (Fig. 13). The BMDc/BMDnc ratios for the NR, STR, and DO groups were 1.38%, 44.27%, and 88.36%, respectively. This also did reveal statistical significance with respect to all groups: NR/STR, $P = 0.0118$; NR/DO, $P < 0.0001$; and STR/DO, $P = 0.0072$ (Fig. 14).

**Histology**

After H&E staining and viewing at $100\times$, the results from each respective group were uniform in appearance. The NR group showed minimal bone remodeling and some osteoblastic activity (Fig. 15). The STR group was noted to have some collagen present...
with increased number of layered osteoblasts parallel to existing bone compared with the NR group (Fig. 16). Lastly, the DO group demonstrated exuberant bone remodeling with increased osteoblasts and organized collagen matrix in longitudinal lines connecting areas of new bone (Fig. 17).

**DISCUSSION**

Designing cleft palate models can be performed either by surgically creating a cleft, as was done in this protocol, or congenitally creating a cleft palate. An animal model in which a congenital cleft palate is created by teratogens given to pregnant mothers would be ideal. There have been several reports of such congenitally created cleft palates. However, there is no way to standardize the size and extent of the cleft palate. An additional disadvantage to the congenitally created cleft palate model is the high risk of additional anomalies that could further affect the health of the animals and therefore standardization of the study.

Surgically created clefts have been studied in several animal models including mice, rabbits, cats, dogs, and sheep. The use of a rabbit model for several phases of study has been performed, investigating the embryology, growth, and effects of palatal, lip, and alveolar surgery. Several of these studies provide detailed cephalometric analysis of cranial growth as a basis for comparison. In addition, several stages of palatogenesis in the NZW rabbit are comparable to human palatal development. We believe that this makes the NZW rabbit model appropriate to investigate the effectiveness of DO on cleft palatal repair.

We propose that DO may have utility in decreasing the rate of oronasal fistula as well as speech disturbances in wide clefts by creating a more normal bony anatomy at the cleft site. It also has the advantage of reducing soft tissue scarring seen with laterally based mucoperiosteal flap closure, although the effect of a palatal osteotomy on midface growth has yet to be further investigated. We evaluated bone density using micro-CT to obtain both a quantitative BMD and content. Micro-CT has been used in previous studies to quantify both bone density and mechanical properties in animal models including rabbit. The use of this technology has also been validated against other common means of measuring bone density including dual energy x-ray, absorptiometry and peripheral quantitative computer tomography. Our results show that there is a significant difference in the density of bone created with DO as compared with either a control NR or STR model. The percentage of cleft to noncleft ratio was also performed to obtain standardization within each micro-CT. Although there was a significant difference in the cleft/noncleft ratio between the DO and the control groups, this may be somewhat misleading. The additional osteotomy created in the noncleft side of the DO group likely affected the bone density ratio. This was further revealed in assessing the decreased BMC and BMD of the noncleft side of the DO group palates compared with the other 2 groups. Therefore, it is prudent to assume that at 9 weeks, this osteotomy site is not be fully ossified, and the ratio of cleft to noncleft density may be skewed.

The process of progressive gap healing to lengthen bone also has the beneficial effect of simultaneous expansion of the soft tissues, including vessels, nerves, muscle, and epithelium. This has been termed distraction histiogenesis. Many of these techniques have been developed as an extension of conventional techniques in children with craniofacial disorders such as cleft lip and palate. We believe that the mechanisms of distraction histiogenesis could prevent the facial growth disturbances often seen in humans in which a standard soft-tissue-only repair has been associated. To
evaluate this, we performed direct cephalometric measurements to evaluate the anterior and posterior maxillary width as well as posterior facial width. Although there are several cephalometric indices, we chose the ones that would most closely correlate to the midface growth deficiencies seen in clinical practice. This study did reveal that there are no significant differences with regard to facial growth between treatment groups. However, there are several limitations in this study that make it difficult to assume conclusively that DO will prevent facial disturbances. First, all cephalometric measurements were performed in a direct fashion on postmortem skulls; therefore, we do not have any preextracted data to compare these data. Also, we chose to use 10- to 12-week-old rabbits because it was at this age that they reached a suitable size and weight to safely perform the surgical intervention and postoperative care. In future studies, we will need to use younger rabbits before much of their skull and midface growth occurs to obtain a more conclusive answer about the effect of an osteotomy and distraction on growth as compared with mucoperiosteal flaps.

The formation of an oronasal fistula is a complication of palate repair—one that is particularly difficult to correct. The incidence of this complication ranges between 3% and 45%. Surgical technique has been found to be one factor influencing the frequency of palatal fistula with Wardill, Furlow, Langenbeck, and Dorrance styles of closure having progressively fewer fistulas. However, possibly more important than type of surgical repair, the preoperative Veau classification may predict which patients are at increased risk of complications such as oronasal fistula. In addition, although several options exist for their closure, none of them are ideal with an overall recurrence rate near 40% depending on the size and location of the fistula. Persistent fistulas can affect speech adversely and are an avenue for food and fluids to enter the nose; they can also complicate the management of the alveolar cleft when it is time for bone grafting. Most fistulas occur anteriorly, or in the postalveolar position. This is a particularly difficult area to correct due to the lack of mobile soft tissue with a healthy blood supply. In some case reports, DO has facilitated the closure of fistulas. Both in the case of an anteriorly located fistula associated with the osteotomy and movement of a palatal tooth and as in a case of a fistula associated with an irradiated palate. DO has been shown to be a potential modality for repair of cleft fistula. If used at the time of primary repair, the new bone generated by DO across the cleft could eliminate the complication of palatal fistula as well as be a mode of therapy for current patients with cleft fistulas.

In conclusion, we believe that this study demonstrated that DO can be safely performed to close a surgically created cleft palate in a rabbit model. This technique has the potential to be successful in treating some of the wider and more severe clefting patterns, which more likely lead to complications. Distraction osteogenesis may also be used as an option to treat complications of palatal surgery, including oronasal fistulas, and reduce tertiary operations. Future studies on younger rabbits will be needed to assess whether DO has a true advantage over soft tissue repair in preventing facial growth disturbances.

ACKNOWLEDGMENTS

We thank Drs Sharon Murphy and Charles Keller of the San Antonio Children’s Cancer Research Institute for donating the micro-CT scans. We also thank Dr Carol A. Van der Harten and Quest Diagnostics, Las Vegas, NV, for their preparation and assessment of the histologic analysis.

REFERENCES