A Comparison of Vascularity, Bone Mineral Density Distribution, and Histomorphometrics in an Isogenic Versus an Outbred Murine Model of Mandibular Distraction Osteogenesis

Edward G. Carey, BA, * *Sagar S. Deshpande, BS,*† *Alexander R. Zheutlin, BS,*‡ *Noah S. Nelson, BS,*§ *Alexis Donneys, MD, MS,*‖ *Stephen Y. Kang, MD,*¶ *Kathleen K. Gallagher, MD,*# *Peter A. Felice, MD,* ** *Catherine N. Tchanque-Fossuo, MD, MS,*†† *and Steven R. Buchman, MD*‡‡

Purpose: The vascularity, bone mineral density distribution, and histomorphometric data between the inbred, isogenic Lewis rat and the outbred, nonisogenic Sprague Dawley rat within mandibular distraction osteogenesis (MDO) were evaluated to allow future researchers to compare the results generated from these 2 animals. We hypothesized that little difference would be found between the 2 strains within these metrics.

Materials and Methods: We implemented a comparative study between the Lewis and Sprague Dawley rat strains within MDO. The sample was composed of 17 male Lewis and 17 male Sprague Dawley rats that underwent surgical external fixation and distraction. The rats' hemimandibles were distracted to a total distance of 5.1 mm. After 28 days of consolidation, 9 rats from each group underwent bone mineral density distribution analysis. The remaining rats from each group were analyzed for the vascular and histologic metrics. Descriptive and bivariate statistics were computed, and the *P* value was set at .05.

Results: We demonstrated successful MDO in all the rats, with no significant difference found in the histologic or bone mineral density distribution metrics. No significant differences were found in any of the vascular metrics, with the exception of vascular separation, which was not normalized to the mandibular volume ($P = .048$).

Conclusions: The results of the present study have demonstrated that little dissimilarity exists between the isogenic Lewis and outbred Sprague Dawley models of MDO. Thus, researchers can confidently

*Medical Student, Craniofacial Research Laboratory, Section of Plastic Surgery, University of Michigan, Ann Arbor, MI.

†Medical Student, Craniofacial Research Laboratory, Section of Plastic Surgery, University of Michigan, Ann Arbor, MI.

‡Medical Student, Craniofacial Research Laboratory, Section of Plastic Surgery, University of Michigan, Ann Arbor, MI.

§Research Fellow, Craniofacial Research Laboratory, Section of Plastic Surgery, University of Michigan, Ann Arbor, MI.

‖Research Fellow, Craniofacial Research Laboratory, Section of Plastic Surgery, University of Michigan, Ann Arbor, MI.

¶Assistant Professor, Department of Otolaryngology–Head and Neck Surgery, The Ohio State University, Columbus, OH.

#Assistant Professor, Department of Otolaryngology–Head and Neck Surgery, Baylor College of Medicine, Waco, TX.

**Resident, Plastic Surgery and Aesthetics Center, University of Florida, Gainesville, FL.

††Research Fellow, Department of Dermatology, University of California Davis, Sacramento, CA.

‡‡Chair, Pediatric Plastic Surgery, Craniofacial Research Laboratory, Section of Plastic Surgery, University of Michigan, Ann Arbor, MI.

Funding was provided by the National Institutes of Health ("Translational Optimization of Bone Regeneration in the Irradiated Mandible"; grant R01 CA12587-06) to S. R. Buchman.

Address correspondence and reprint requests to Dr Buchman: Pediatric Plastic Surgery Section, University of Michigan, 4-730, CS Mott Children's Hospital, 1540 East Hospital Drive, Ann Arbor, MI 48109-0219; e-mail: sbuchman@med.umich.edu

Received January 25 2016

Accepted April 18 2016

0278-2391/16/30116-1

http://dx.doi.org/10.1016/j.joms.2016.04.016

compare the gross results between the 2 strains, with consideration of the very small differences between the 2 models. For studies that require an isogenic strain, the Lewis rat is an apt surrogate for the Sprague Dawley strain.

J Oral Maxillofac Surg ■:1-11, 2016

Mandibular defects can arise from a wide array of congenital and acquired conditions, such as Treacher-Collins syndrome, osteoradionecrosis, benign and malignant neoplasms, and bone loss secondary to trauma.[1-3] Reconstruction of these deficiencies has been most often accomplished using bone grafting and free tissue transfer with rigid fixation.[4] However, this approach has considerable shortcomings. This onerous operation places the patient at risk of substantial donor site morbidity.[2] Furthermore, rigid fixation is especially precarious in the pediatric population owing to the potential injury to developing dentition and the possible migration of fixation hardware in the immature mandible.[5] Finally, bone grafts have been associated with relapse and reoperation in part because of the physiologic forces of mastication that impart a significant amount of strain on the graft.[6] As such, it benefits the reconstructive surgeon to be armed with alternative techniques that can be used to evade these challenges.

Distraction osteogenesis (DO) is a powerful operative modality in which regenerate bone is created through the gradual separation of opposing osteogenic fronts. Although originally developed as a bone-lengthening procedure, reconstructive surgeons routinely use DO for a broad range of conditions, including hemifacial microsomia, maxillary hypoplasia, craniosynostosis, and conditions requiring alveolar ridge augmentation.[7-10] Mandibular DO (MDO) possesses an advantage over free tissue transfer in mandibular reconstruction in that it avoids donor site morbidity by generating bone and soft tissue from the local endogenous substrate. Despite its strong reconstructive potential, the procedure has several drawbacks. The length of time required for the distracted bone to consolidate has been a limiting factor for its widespread use.[11] Additionally, the diminution of the local regenerative milieu imparted by radiation has precluded the widespread use of MDO within patients with head and neck cancer who have mandibular defects secondary to tumor extirpation.[12,13] Given its potency as a reconstructive modality, much can be gained from optimizing the procedure within the laboratory setting to address these limitations.

Our laboratory has tremendous experience investigating MDO, having developed a novel murine model more than a decade ago.[14] We investigated several therapeutic interventions that markedly improved MDO within this model. Specifically, our laboratory has demonstrated the cytoprotective effects of amifostine within the distracted, irradiated mandible.[15] Additionally, we have shown that the vasculogenic effects of deferoxamine bolster healing within the distracted mandible shortens the consolidation period substantially.[16,17] Until recently, these studies were conducted solely in the outbred Sprague Dawley rat strain. Our laboratory has since investigated mesenchymal stem cell implantation to augment healing within MDO.[18] For studies using stem cell therapy, the use of an outbred strain is suboptimal. A host-versus-graft immune response would almost certainly occur at the site of implantation if an allograft were to be used within an outbred strain. Although an allograft could be used in immunocompromised rats, such as athymic strains, infection is a major concern with any surgical procedure. Also, the inflammatory pathways, extensively involved in bone healing and regeneration, are altered in athymic rats.[19-21] An alternative approach would be to use an autologous stem cell transfer. However, this can be technically impractical in small animal models, because it is often extremely challenging to attain a sufficient volume of bone marrow without subjecting the rat to substantial morbidity. Therefore, an immunocompetent, isogenic strain of rat large enough to undergo an arduous surgery and also allow for the seamless procurement and transfer of tissue would provide an ideal experimental animal model to deftly investigate the utility of stem cells to enhance MDO. The inbred Lewis strain was chosen by our laboratory as the best isogenic option, because it is the largest and fastest growing of any available isogenic rat strain.

Studies undertaken within the Lewis strain have provided substantial evidence that stem cell therapy can enhance MDO.[22] The implantation of stem cells improves the bony union rates and the vascular, radiomorphometric, and biomechanical strength metrics in the distracted, irradiated Lewis mandible.[22,23] Although the power of stem cell therapy in MDO is clear, these studies cannot be reliably compared with studies undertaken using the Sprague Dawley rat until it has been established that MDO progresses similarly within these 2 strains. Although a previous study demonstrated substantial similarity between the 2 strains within radiomorphometric and biomechanical response parameters after MDO, no