Effects of lactoferrin on bone resorption of midpalatal suture during rapid expansion in rats

Ye Cheng,[a] Jianfeng Sun,[b] Zeyuan Zhou,[c] Jie Pan,[c] Shujuan Zou,[c] and Jianwei Chen[c]
Chengdu, Nanjing, and Ningbo, China

Introduction: The aim of this study was to investigate the effect of lactoferrin (LF) on bone resorption of rats' midpalatal sutures during rapid palatal expansion. **Methods:** Sixty male 5-week-old Wistar rats were randomly divided into 3 groups: expansion only (EO), expansion plus LF (E + LF), and sham device (control). **Results:** Microcomputed tomography showed that the bone volume/tissue volume ratio and the relative bone mineral density of the suture bone were significantly increased in the E + LF group compared with the EO group. Histochemical staining suggested that the activity of osteoblast-like cells and the amount of new bone formation were stimulated in the E + LF group whereas the activity of osteoclasts showed no obvious difference between groups. On the other hand, the immunohistochemical and the real-time polymerase chain reaction results showed that the expressions of receptor activator of nuclear factor kappa B ligand and osteoprotegerin had no significant difference between the EO and E + LF groups. **Conclusions:** These findings demonstrated that LF could stimulate bone volume and bone density in midpalatal sutures during the suture remodeling process under tensile force. However, this enhancement effect was not caused by the reduction of bone resorption. (Am J Orthod Dentofacial Orthop 2018;154:115-27)

Rapid palatal expansion (RPE) is a widely used approach to correct transverse maxillary deficiency. Stretching of the sutures induces a biologic chain of events that leads to new bone deposition in the midpalatal suture.[1,2] During the procedure, the suture undergoes remodeling, which includes bone resorption and formation and fiber rearrangement.[3] The suture remodeling continues until the architectural environment achieves equilibrium. Although the midpalatal suture can be successfully expanded, relapse has often been reported.[4] A major reason for early relapse is inadequate bone formation in the suture.[5] Therefore, enhancing bone formation and inhibiting bone resorption in the midpalatal suture may improve the stability of RPE.

Lactoferrin (LF) is an iron-binding glycoprotein that belongs to the transferrin family with pleiotropic functions including antimicrobial and immunomodulatory activities. LF is present in high concentrations in colostrum and milk and circulates at concentrations of 2 to $7 * 10^{-6}$ g per milliliter in the human body. Recently, there has been growing interest in the potential use of LF for the improvement of bone metabolism.[6] Investigations have shown that LF could not only induce proliferation of primary osteoblasts, increase osteoblast differentiation, and protect osteoblastic cells from apoptosis, but also potently inhibit osteoclastogenesis, thus reducing the number of cells that can actively resorb bone.[7] The numbers of newly developed osteoclasts in mouse bone marrow cultures, assessed as multinucleated cells staining positive for tartrate-resistant acid phosphatase, were significantly decreased due to LF at concentrations of 10 μg per milliliter; at 100 μg per milliliter, osteoclastogenesis was completely arrested.[8] Taken together, LF might cause an overall increase in bone mass by promoting bone formation and inhibiting bone resorption.

[a]State Key Laboratory of Oral Diseases and Department of Orthodontics, West China Hospital of Stomatology, Sichuan University, Chengdu, China; Nanjing Stomatological Hospital, Medical School of Nanjing University, Nanjing, China.
[b]Ningbo Dental Hospital, Ningbo, China.
[c]State Key Laboratory of Oral Diseases and Department of Orthodontics, West China Hospital of Stomatology, Sichuan University, Chengdu, China.
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Address correspondence to: Jianwei Chen, State Key Laboratory of Oral Diseases and Department of Orthodontics, West China Hospital of Stomatology, Sichuan University, 14 Section 3 Ren Min Nan Lu, 610041 Chengdu, China; e-mail, bulb13@163.com.
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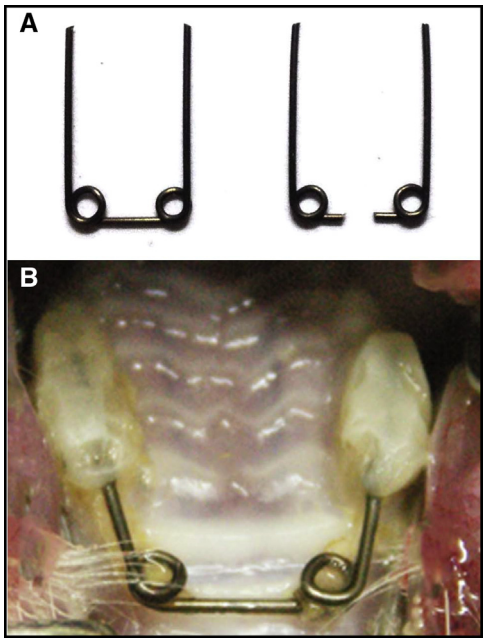

Fig 1. Mechanical expansion devices used in this study: **A,** the expansion devices applied in the EO and E + LF groups (before activation) and control group (separated at the middle); **B,** intraoral view of the expansion device in the rat.

Receptor activator of nuclear factor kappa B ligand (RANKL), receptor activator of nuclear factor kappa B (RANK), and osteoprotegerin (OPG) system is a critical signal transduction pathway that regulates osteoclastogenesis.[9] RANKL is a new tumor necrosis factor-family molecule that is preferentially expressed on the cell membrane of committed preosteoblastic cells, whereas its specific receptor, RANK, is expressed in osteoclast progenitors. RANKL and RANK bind with each other through cell-to-cell contact between preosteoblasts and osteoclast progenitors, and subsequently activate osteoclastogenesis. The interaction between RANKL and RANK is regulated by OPG, a decoy receptor for RANKL also produced by osteoblast cells, which binds with RANKL competitively and blocks RANKL-RANK signaling; therefore, it inhibits osteoclastogenesis.[10] Previous studies have shown that RANKL is a key osteoclast differentiation factor required for osteoclast development and bone remodeling in vivo, and the biologic effects of OPG on bone cells include the inhibition of terminal stages of osteoclast differentiation, suppression of the activation of mature osteoclasts, and induction of their apoptosis.[11,12] The balance of the counteraction between RANKL and OPG regulates the development and activation of osteoclasts and bone metabolism. Therefore, the ratio of RANKL and OPG is determined to regulate osteoclast activity and bone metabolism.[13,14]

Therefore, we supposed that LF might inhibit osteoclastogenesis in vivo, and the critical signaling pathway regulating osteoclastogenesis—the OPG/RANK/RANKL system—plays an important role in this process. The main purpose of this study was to investigate the effect of LF on bone resorption during midpalatal suture remodeling under tensile force.

MATERIAL AND METHODS

Sixty 5-week-old male Wistar rats weighing 100 ± 10 g were obtained from the experimental animal center at Sichuan University, Chengdu, China. The rats were fed the same ground diet with fresh drinking water, and their health was checked daily. All study procedures were approved by the institutional animal care and use committee of Sichuan University.

The animals were randomly divided into 3 groups of 20 animals each: expansion only (EO), expansion plus LF (E + LF), and sham device (control). The rats in the EO and E + LF groups were subjected to rapid mechanical expansion, and those in the control group received expansion devices separated at the middle (no activation) (Fig 1, *A*). An expansion spring with 2 helices was fabricated with 0.014-in orthodontic wire (supreme type; AJ Wilcock Australian Wire, Birmingham, United Kingdom). The initial expansion force was calibrated to 50 ± 5 g. The spring was fitted between the maxillary right and left molars and secured by Transbond LR light-cured resin (3M Unitek, Monrovia, Calif) (Fig 1, *B*). The animals were under anesthesia with a combination of ketamine (87 mg/kg) and xylazine (13 mg/kg) during setting of the appliances. The fit was checked daily; in the EO and E + LF groups, the appliances were activated immediately after bonding. No reactivation was performed during the experimental period. Animals in the E + LF group were gavage-fed daily with 1 mg per 100 g of body weight of LF (95%; Westland Milk Products, Hokitika, New Zealand) dissolved in saline solution at a concentration of 1000 μg per milliliter. The nimals in the other 2 groups received the same volume of vehicle (saline solution). Five animals from each group were randomly selected and killed 1, 4, 7, and 14 days after bonding of the spring.

The maxilla including the midpalatal suture of each animal was dissected. For microcomputed tomography (μCT) evaluation, the specimens were fixed in 4% paraformaldehyde buffer for 48 hours at 4°C. For histochemical and immunohistochemistry staining, fixed tissue sections were decalcified in neutral 10% ethylene