Midpalatal Suture of Osteopetrotic (op/op) Mice Exhibits Immature Fusion

Toshitsugu KAWATA, Chiyoko TOKIMASA, Tadashi FUJITA, Seiki KAWASOKO, Masato KAKU, Hiroki SUGIYAMA, and Kazuo TANNE

Department of Orthodontics, Hiroshima University School of Dentistry, 1–2–3 Kasumi, Minami-ku, Hiroshima 734-8553, Japan

Abstract: The midpalatal suture was observed histologically in both toothless osteopetrotic (op/op) and normal (control) mice. The normal mice had a mature sutural structure, which consists of a well-developed cartilage cell zone and palatal bone. In contrast, the thickness of the cartilage cell zone was substantially greater in the op/op mice than that in the controls. Moreover, the cartilage cells in the op/op mice were frequently found in the palatal bone as well as in the sutural space, exhibiting an imperfect fusion. It seems that immature fusion at the sutural interface in the op/op mice is related to a decrease in biting or masticatory force accompanied by the failure of tooth eruption in addition to an essential defect in osteoclast differentiation, which is a congenital symptom in op/op mice.

Key words: histomorphology, midpalatal suture, op/op mouse

Osteopetrotic mutant mice (op/op) is characterized by systemic bone sclerosis, deformation of the skull and jaw, and failure of tooth eruption due to defect in bone resorption [4–6, 13, 14, 17, 20]. The op/op mice have a prominent deficiency of osteoclasts, monocytes and peritoneal macrophages [13, 14, 21, 22]. It was also reported that an injection of purified recombinant human M-CSF (rhM-CSF) elicited osteoclast differentiation and cured the bone sclerosis and failure of tooth eruption in the mutant mouse [2, 3, 10, 18, 19].

The op/op mouse, meanwhile, has been used as a model of the “toothless” mouse in morphological and physiological studies to investigate the relationship between mastication, masseter muscle development [12, 15] and condylar growth [7, 8]. Longitudinal cephalometric analysis also indicated that changes in masticatory function affected the anterior facial height as well as the local bone morphology at the muscle attachment area [9]. Effects of diet of low physical consistency were revealed for the transverse underdeveloped width of the maxilla in growing rats [1, 16, 20]. In view of these considerations, these mutant mice may serve as an experimental model to investigate the effects of osteoclast deficiency on bone remodeling in the craniofacial skeleton and matters relevant to the masticatory system.

The present study was to examine the histological changes in the midpalatal suture in mutant mice and to
assess the role of mechanical stimuli from mastication in the growth of midpalatal suture.

Osteopetrotic (op/op) mice and control littermate (normal) mice were obtained from B6C3F1-w/a-op/+ breeding pairs (Jackson Laboratory, Bar Harbor, ME). The mice were kept in metal cages (22 × 32 × 11 cm) with autoclaved wood chips for bedding in an animal room (temperature; 24 ± 2°C, relative humidity; 50 ± 5%). Newborn male mice were weaned in 15–20 days. Homozygous recessive op/op mice were identified by failure in tooth eruption and a characteristic domed skull 10 days after birth. The op/op mice were fed a granulated diet, and normal mice were fed a solid diet (CE2: Clea Japan, Tokyo, Japan). Five op/op mutants and five normal mice were sacrificed for histological examination at 10 and 30 days old. The upper jaws removed from the skulls were fixed in 4% formaldehyde for 12 hr at 4°C, decalcified in 5% ethylenediamine tetraacetic acid (EDTA) (pH. 7.4) for one week, embedded in paraffin, and cut into frontal sections 7 μm thick. These sections were stained alternately with hematoxylin and eosin (HE) and examined under a light microscope (BH; Olympus, Tokyo, Japan).

At the age of 10 days: The anterior palatal foramen seemed to be longer in the op/op mice than that in the normal mice (Figs. 1A and 1C). The midpalatal suture in the normal mice had a typical cartilaginous growth plate structure composed of clear zones of cell multiplication, hypertrophy and matrix calcification (Fig. 1B). On the other hand, in the midpalatal suture of op/op mice, the cartilage cell layer was thicker and exhibited an irregular hypertrophic cell zone without ossification (Figs. 1C and 1D). Moreover, intra-cartilaginous ossification was not observed in the 10-day-old op/op mice. The palatal bone had a wide bone marrow cavity in the normal mice (Figs. 1A and 1B). In contrast, the cartilage cell zone was thicker in the op/op mice than that in the controls (Figs. 1B and 1D). Moreover, the cartilage cells in the op/op mice were frequently found in the palatal bone as well as in the sutural interface (Figs. 1C and 1D). Accordingly, the op/op mice presents immature fusion at the midpalatal suture in the op/op mice a congenital disease, closely linked to inferior bone metabolism was considered. Loevy [11] suggested immature fusion of the palatal plate as a cause of cleft palate. It is therefore possible that the op/op mice have a cleft palate.

At this time, the molars remained within the bony crypts in both the op/op and normal mice. Moreover, the normal and op/op mice showed no significant differences in the shape of the alveolar bone or the position of the molars (Figs. 1A and 1C). Since this age is in the period before weaning the mice, the masticatory function of both the normal and op/op mice was assumed to be at the same developmental stage.

At the age of 30 days: Histologically the midpalatal suture in the normal mice had a typical cartilaginous growth plate structure composed of cell multiplication, hypertrophy and matrix calcification. The thickness of the palatal suture area was greater in the normal mice than in the op/op mice (Figs. 2A and 2C). The palate bone of the normal mice became much thicker than in the op/op mice (Figs. 2B and 2D). On the other hand, in the op/op mice, the palatal bone adjacent to the midpalatal suture was still composed of a cartilagenous structure (Fig. 2D). Cartilaginous ossification was observed close to the oral cavity in the palatal bone of the op/op mice (Fig. 2D), but close to the nasal cavity there was observed no cartilaginous ossification in the palatal bone of the op/op mice (Fig. 2D). These findings suggested that the mechanical stimulus from mastication also played an important role in the morphogenesis of the palatal bone toward the mouth in the initial ossification of the midpalatal cartilage. As the morphological characteristics of the bones in the op/op mice indicate bone sclerosis and/or hyperostosis [14], it became difficult to obtain satisfactory results in the present study.

At this age, the molars remained within their bony crypts in the op/op mice (Fig. 2C). On the other hand, the molars exhibited well-developed roots and periodontal ligament in the normal mice (Fig. 2A).

In conclusion, it seems that immature fusion at the sutural interface of the op/op mice may be related with the decrease in bite force due to non-eruption of the molars in the upper jaw, in addition to a congenital defect in osteoclast differentiation in the op/op mice.

Acknowledgments

The authors are grateful to the Research Facilities for Laboratory Animal Science, Hiroshima University School of Medicine.
Fig. 1. Microphotographs of midpalatal suture of 10-day-old normal (A, B) and op/op (C, D) mice. A, C: HE staining. ×65. B, D: HE staining. ×292.

Fig. 2. Microphotographs of midpalatal suture of 30-day-old normal (A, B) and exp/log (C, D) mice. Numerous cartilage cells are seen close to the nasal cavity in the palate bone (arrowheads) A, C: HE staining. × 65. B, D: HE staining. × 292.
References