Original Article

Effects of Mandibular Advancement plus Prohibition of Lower Incisor Movement on Mandibular Growth in Rats

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ABSTRACT

Introduction: To test the hypothesis that mandibular advancement with the use of a fixed functional appliance combined with prohibition of labial movement of the lower incisors will have no effect on mandibular growth in growing rats.

Materials and Methods: Fifteen 4-week-old male rats were divided into fixed, unfixed, and control groups (n=5, each). Bite-jumping appliances were used in the fixed and unfixed groups. Sites of bone perforation and the lower incisors were connected with ligature wires in the fixed group. The ramus height, mandibular length, and inclination of lower incisors were examined for 4 weeks, and those values were compared among five intervals and three groups by through one-way analysis of variance models and the Bonferroni multiple comparison test for post hoc comparison. **Results:** Increases in ramus height and mandibular length during the experimental period were 1.5 mm and 2.5 mm in the fixed group, 1 mm and 1.5 mm in the unfixed group, and 1.2 mm and 1.9 mm in the control group, respectively. Growth of ramus height and growth of mandibular length in the fixed group were greater than in the unfixed and control groups during the experimental period. The inclination of lower incisors in the unfixed group was increased 8.0 degrees throughout the experimental period, which differed from results obtained in the other groups.

Conclusions: Mandibular growth was accelerated effectively before and during the pubertal period in rats by mandibular advancement with a fixed functional appliance combined with prohibition of labial movement of the lower incisor. (*Angle Orthod.* 2009;79:1095–1101.)

KEY WORDS: Fixed functional appliance; Acceleration of mandibular growth; Skeletal anchorage

INTRODUCTION

Functional appliance therapy in growing patients can correct Class II malocclusion with recessive mandibular growth by acceleration of mandibular growth and dental changes. 1-3 Two types of functional appliances are available: removable appliances and fixed

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appliances. It has been suggested that fixed functional appliances are more effective than removable ones for the acceleration of mandibular growth during treatment of maxillary protrusion because of their full-time action without patient compliance.⁴

According to a previous study, overjet correction by a fixed functional appliance was mainly the result of an increase in mandibular length (48%) and labial movement of lower incisors (35%),¹ with the lower incisors showing a definite proclination because of the force vectors involved with the fixed functional appliances.^{2,3} Therefore, it can be speculated that greater skeletal improvement is possible with fixed functional appliance therapy combined with prohibition of labial movement of the lower incisors in treating maxillary protrusion when compared with conventional functional appliance therapy.

In a recent study on the effect of mandibular advancement with a fixed functional appliance in growing rats,^{5–7} it was reported that mandibular growth was accelerated by new bone formed in condyle. However, the influence of labial movement of the lower incisor

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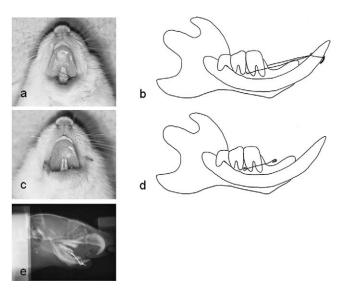


Figure 1. Fixation of the lower incisors by a ligature wire. a, b, and e: The lower incisors and the bone perforation sites on the alveolar bone between the mandibular first and second molars on the right and left sides were connected with ligature wires (φ 0.25 mm) in the fixed group. c and d: Ligature wires were ligatured only at bone perforation sites in the unfixed group.

on the acceleration of mandibular growth when the fixed functional appliance is used is unclear.

A skeletal anchorage system that used implant anchors such as titanium screws and miniplates provides absolute anchorage during orthodontic treatment, 8-10 and enables an en masse retraction without undesirable anchorage loss. 10,11 Therefore, if such a skeletal anchorage system is used on the lower incisors during functional appliance therapy, the labial movement of the lower incisors that occurs with use of the appliances could be prevented; therefore, it can be speculated that greater skeletal improvement is possible with fixed functional appliance therapy. The purpose of the present study was to investigate the effects of mandibular advancement with a fixed functional appliance combined with prohibition of labial movement of the lower incisor on mandibular growth in growing rats.

MATERIALS AND METHODS

Animals

A total of 15 male Wister rats (weight, 70 to \sim 80 g; age, 4 weeks old) were used in the present study. The

animals were caged individually with regulated light, temperature, and humidity at the University Frontier Science Research Organization Center. They were fed a soft diet to prevent any damage to the orthodontic appliances. Experimental conditions and procedures in the present study were approved by the Animal Ethics Committee of Kagoshima University (#199).

All experimental procedures were performed under interperitoneal anesthesia with sodium pentobarbital (0.012 mL/g). The 15 rats were divided randomly into two experimental groups and one control group (five rats in each group). A bite-jumping appliance was fitted to the upper incisors of each rat and was worn all day in the two experimental groups. The appliance was designed so that the mandible could be located 3.5 mm anteriorly and 3 mm inferiorly by contact with the lower incisors (Figure 1a,c).6 The appliance was cemented bilaterally to the whole surfaces of acidetched upper incisors with Super-Bond resin cement (Sun Medical, Shiga, Japan). Bone perforations were made on the mandibular alveolar bone with a fissure burr (#008) in the two experimental groups and at the alveolar bone edge areas between the first and second molars of the right and left sides, to avoid injury to the inferior alveolar nerves and the roots of the lower incisors.

In the fixed group, the bone perforation sites and the lower incisors were connected with ligature wires (0.25 mm) passively to avoid labial movement of the lower incisors that may occur when the bite-jumping appliance is used (Figure 1a,b,e). The ligature wires for the lower incisors were bonded with Super-Bond resin cement to avoid looseness of the ligature wires. In the unfixed group, the same ligature wires were ligatured only at the same sites on the right and left sides, to meet all experimental conditions, excluding inhibition of labial movement of the lower incisors between the two experimental groups (Figure 1c,d). No significant differences were noted among the three groups in terms of weight of rats before and after the experiment (Table 1).

Cephalometric Analysis

Cephalometric radiographs were taken immediately before the experiment (T0) and at 1 week (T1), 2 weeks (T2), 3 weeks (T3), and 4 weeks (T4) after com-

Table 1. Body Weight of Rats Before and After the Experiment

	Fixed Gro	Fixed Group (n = 5)		oup (n = 5)	Control Gro		
	Mean	SD	Mean	SD	Mean	SD	
Before experiment, g	75.4	9.1	73.3	6.8	75.4	10.6	NS*
After experiment, g	197.3	36.3	170.6	62.5	185.4	76.8	NS

^{*} NS, Not significant (ANOVA and Bonferroni test).

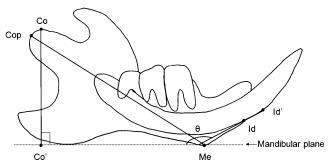


Figure 2. Cephalometric measurement points and lines used in this study: Co, The most superior point of the condyle; Co', the crossing point between the extension of the mandibular plane and the perpendicular of the mandibular plane from Co; Cop, the most posterior point of the condyle; Me, the most inferior point on the labial alveolar bone contour; Co-Co', ramus height; Cop-Me, mandibular length. Measurement of the inclination of the lower incisors: Id, The most anterior point on the labial alveolar bone; Id', a point 2.8 mm distant from Id on the labial surface; θ , angle of the Id-Id' line to the mandibular plane (inclination of the lower incisor).

mencement of the experiment and were traced by one experienced orthodontist. A film packet was placed 10 cm from the center of the head of the rat. The device was placed on the floor with a 110 cm object-to-film distance (10 mA, 60 kVp, 4 seconds) and the central ray perpendicular to the head of the rat. The distance between the radiographic focus and the film was 110 cm, and between the midsagittal plane and the film 10 cm, producing a magnification of 10%.

Co, Co', Cop, and Me were used to measure the ramus height (Co-Co') and mandibular length (Cop-Me) of the rat (Figure 2).12,13 In addition, the most anterior point on the labial alveolar bone (Id)14 and the point that was 2.8 mm distant from the Id on the labial surface (Id') were used to measure the inclination of the lower incisors. These points were selected primarily for three reasons: (1) According to previous studies, 15,16 the circumference of the lower incisor of the rat is established in the basal tooth part, and, once established, it remains constant; (2) the line from Id to Id' was an almost straight line on the labial surface in all rats; and (3) according to our pilot study (n = 5), the coefficient of variation of the measurement values in the angle of the Id-incisal edge line to the mandibular plane was significantly larger than in the angle of the Id-Id' line to the mandibular plane (Wilcoxon signed ranks test; P = .043). Therefore, we measured the angle of the mandibular plane to the Id-Id' line to examine the inclination of the lower incisors. Cephalometric measurement points were established and digitized by computer-assisted image-analyzing software; (developed at the US National Institutes of Health).

The causal error was calculated according to Dahlberg's formula (Se² = $\Sigma d^2/2n$), where Se² is the error

variance, d is the difference between the repeated measurements, and n is the number of double measurements made. The According to Dahlberg's formula, the mean error ranged from 0.039 to 0.041 mm for linear measurements and 0.28 degrees for angular measurements.

Statistical Analysis

Changes in ramus height, mandibular length, and inclination of the lower incisors from T0 to T1 (T0-1), T1 to T2 (T1-2), T2 to T3 (T2-3), T3 to T4 (T3-4), and T0 to T4 (T0-4) were calculated. One-way analysis of variance models (ANOVA) and the Bonferroni multiple comparison test for post hoc comparison were used to determine the significance of differences among the five intervals or the three groups. Tests were performed with the use of statistical analysis software (Statistical Package for the Social Sciences [SPSS], version 14; SPSS Inc, Chicago, III).

With regard to sample size, we carried out a sample size calculation¹⁸ using data derived from our pilot experiment with nine rats. The maximum difference of the mean Cop-Me score among fixed, unfixed, and control groups was 2.01 (SD, 0.09). From these data, with a standardized difference of 1.05, a sample size of three rats in each group would yield a power of 0.80 with a significance level of .05.

RESULTS

Measurement values of amount and rate of growth in mandibular length, ramus height, and inclination of the lower incisors at each interval are shown in Tables 2, 3, and 4.

In the fixed group, the growth in Cop-Me and Co-Co' at T2-3 was significantly greater than at the other intervals, and the growth at T1-2 was significantly smaller than at the other intervals (Figures 3 and 4). In the unfixed group, the growth of Cop-Me and Co-Co' at T0-1 was significantly greater than at the other intervals, and the growth at T2-3 was significantly greater than at T1-2 and T3-4. The growth of Cop-Me at T1-2 was significantly smaller than at the other intervals. In controls, the growth in Cop-Me and Co-Co' at T2-3 was significantly greater than at the other intervals, and the growth at T3-4 was significantly greater than at T0-1 and T1-2.

The growth of Cop-Me and Co-Co' at T0-1 in the fixed and unfixed groups was significantly greater than in the control group, and the growth at T2-3 and T3-4 in the fixed and control groups was significantly greater than in the unfixed group (Figures 5 and 6). The growth of Cop-Me and Co-Co' in the fixed group was greater than in the unfixed and control groups at T0-4. Significant differences were noted between the

Table 2. Measurement Values of Amount and Rate of Growth in Mandibular Length (Cop-Me) at Each Interval

	F	Fixed Gro	oup (n = 5)	U	nfixed Gr	oup (n =	5)	Control Group (n $=$ 5)			
	Mean	SD	Mina	Maxª	Mean	SD	Min	Max	Mean	SD	Min	Max
Amount of gro	owth, mm											
T0-1	0.6	0.0	0.6	0.7	0.7	0.0	0.6	0.7	0.1	0.0	0.0	0.1
T1-2	0.1	0.0	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.0	0.1	0.2
T2-3	1.1	0.2	0.9	1.4	0.4	0.0	0.4	0.5	1.1	0.2	0.8	1.3
T3-4	0.6	0.1	0.5	0.7	0.3	0.0	0.3	0.4	0.6	0.1	0.4	0.6
T0-4	2.5	0.2	2.2	2.7	1.5	0.1	1.4	1.6	1.9	0.3	1.4	2.1
Rate of growt	h, %⁵											
T0-1	3.6	0.2	3.4	3.8	3.9	0.1	3.9	3.9	0.6	0.3	0.1	0.8
T1-2	0.6	0.2	0.4	0.8	0.7	0.2	0.4	0.8	0.7	0.2	0.4	0.9
T2-3	6.2	0.9	5.0	7.4	2.5	0.3	2.3	2.8	6.2	0.3	4.5	7.2
T3-4	3.3	0.4	2.6	3.6	1.8	0.2	1.6	1.9	3.2	0.6	2.3	3.5
T0-4	14.3	1.2	13	15.2	9.0	0.4	8.8	9.3	11.1	1.7	8.5	12.1

^a Min, minimum; Max, maximum.

Table 3. Measurement Values of Amount and Rate of Growth in Ramus Height (Co-Co') at Each Interval

	ı	Fixed Group (n = 5)				nfixed Gr	oup (n =	5)	Control Group (n = 5)			
	Mean	SD	Mina	Maxª	Mean	SD	Min	Max	Mean	SD	Min	Max
Amount of gro	owth, mm											
T0-1	0.4	0.0	0.3	0.4	0.4	0.0	0.4	0.5	0.1	0.0	0.0	0.1
T1-2	0.1	0.0	0.1	0.1	0.1	0.0	0.1	0.2	0.1	0.0	0.1	0.2
T2-3	0.6	0.1	0.5	0.7	0.2	0.1	0.2	0.4	0.6	0.2	0.4	0.8
T3-4	0.4	0.1	0.3	0.4	0.2	0.0	0.1	0.2	0.3	0.1	0.3	0.4
T0-4	1.5	0.2	1.2	1.6	1.0	0.1	0.9	1.1	1.2	0.2	0.9	1.5
Rate of growt	h, % ^b											
T0-1	4.5	0.5	4.0	4.8	5.3	0.5	4.7	5.7	1.1	0.3	0.6	1.3
T1-2	1.2	0.3	1.0	1.5	1.6	0.2	1.3	1.9	1.6	0.4	1.1	2.1
T2-3	6.8	0.6	6.4	7.3	2.8	1.0	2.1	4.7	6.8	2.0	5.4	8.6
T3-4	4.0	0.7	3.1	4.5	2.1	0.4	1.6	2.6	3.7	0.7	3.0	4.3
T0-4	17.6	1.8	15.4	18.2	12.2	1.0	11.5	13.9	13.6	3.1	11.8	16.3

^a Min, minimum; Max, maximum.

Table 4. Measurement Values of Amount and Rate of Inclination of Lower Incisors at Each Interval

		Fixed Group (n = 5)				nfixed Gr	oup (n =	5)	Control Group (n = 5)			
	Mean	SD	Mina	Maxª	Mean	SD	Min	Max	Mean	SD	Min	Max
Amount of inc	clination, degre	ees										
T0-1	0.1	0.5	-0.6	0.5	2.1	1.2	1.1	3.6	0.3	0.5	-0.6	0.9
T1-2	-0.2	0.3	-0.7	0.1	2.5	1.8	0.2	4.2	0.0	0.4	-0.5	0.3
T2-3	0.2	0.3	-0.1	0.5	1.7	1.1	0.9	3.5	0.2	0.4	-0.2	0.9
T3-4	-0.2	0.4	-0.8	0.3	1.7	0.6	1.0	2.5	-0.1	0.2	-0.3	0.1
T0-4	0.0	0.4	-0.4	0.6	8.0	2.7	4.1	11.3	0.3	0.8	-0.6	1.5
Rate of inclina	ation, %b											
T0-1	0.1	0.3	-0.4	0.3	1.5	0.9	0.8	2.5	0.2	0.4	-0.4	0.6
T1-2	-0.1	0.2	-0.5	0.1	1.7	1.3	0.1	2.9	0.0	0.3	-0.4	0.2
T2-3	0.2	0.2	-0.1	0.3	1.1	0.7	0.6	2.3	0.2	0.3	-0.1	0.6
T3-4	-0.1	0.2	-0.6	0.2	1.1	0.4	0.7	1.6	-0.1	0.2	-0.2	0.1
T0-4	0.0	0.3	-0.3	0.4	5.5	1.9	2.9	7.6	0.3	0.6	-0.4	1.0

^a Min, minimum; Max, maximum.

^b Rate of growth = Amount of growth at Ti-(i+1)/Mandibular length at $Ti \times 100$ (i=0, 1, 2, 3).

^b Rate of growth = Amount of growth at Ti-(i+1)/Mandibular length at $Ti \times 100$ (i=0, 1, 2, 3).

^b Rate of inclination = Amount of inclination at Ti-(i + 1)/Mandibular length at $Ti \times 100$ (i = 0, 1, 2, 3).

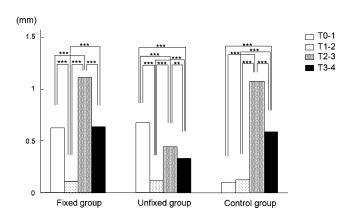


Figure 3. Changes in mandibular length (Cop-Me). **P < .01; ***P < .001.

fixed group and the other two groups in terms of growth of Cop-Me, and between the fixed group and the unfixed group in terms of growth of Co-Co' at T0-4 (Tables 2 and 3; Figures 7 and 8).

Although no changes in the inclination of the lower incisors were observed during the experimental period in the fixed and control groups, the inclination significantly increased throughout the experimental period in the unfixed group (Table 4; Figures 9 and 10).

DISCUSSION

Mandibular growth at T2-3 and T3-4 was significantly greater than at T0-1 and T1-2 in the control group. It has been reported that the peak of mandibular growth is observed at 45 to 50 days of age in rats. 19,20 In the present study, the age of rats during the period from T2 to T4 was 42 to 56 days. Therefore, it is considered that the peak of mandibular growth in rats occurs from T2 to T4 if an orthodontic appliance is not used.

The mandibular growth at T0-1 in the fixed and unfixed groups was significantly greater than at T0-1 in the control group. It is well known that the mandibular condyle is a principal growth site in the mandible.^{4,5,21} It has been suggested that forward and downward

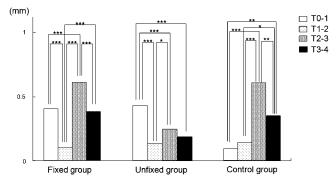


Figure 4. Changes in ramus height (Co-Co'). *P < .05; $^{**}P$ < .01; $^{***}P$ < .001.

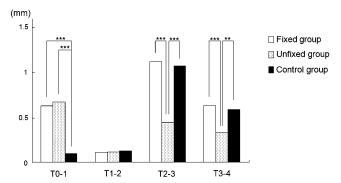


Figure 5. Changes in mandibular length (Cop-Me) in each group. $^{**}P < .01$: $^{***}P < .001$.

mandibular positioning produces new bone in the posterior and middle regions of the glenoid fossa and condyle in growing rats. 5,6 The bite-jumping appliance used in the present study made the mandible move 3.5 mm forward and 3 mm downward. Therefore, it is considered that continuous mandibular advancement by a bite-jumping appliance accelerates mandibular growth before the pubertal growth period. Mandibular growth in the fixed and unfixed groups at T1-2 was minimal growth during the experimental period. However, in the control group, the growth at T0-1 was small, similar to the growth at T1-2, because mandibular growth from T0 to T2 was the growth that occurred before the pubertal period. 19,20 Therefore, it was conceived that mandibular growth in the fixed and unfixed groups at T1-2 seemed to be minimal because T1-2 occurred before the pubertal period.

Mandibular growth at T2-3 in the unfixed group was significantly less than at T2-3 in the fixed and control groups. The circumference of the lower incisor of the rat is established in the basal tooth part, and, once established, it remains constant. ^{15,16} No changes were observed in the inclination of the lower incisors in the control group of the present study. However, the inclination of the lower incisors increased throughout the experimental period in the unfixed group. A study that used rats found that new bone formation in the con-

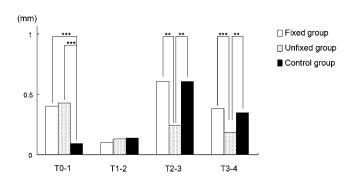


Figure 6. Changes in ramus height (Co-Co') in each group. **P < .01: ***P < .001.

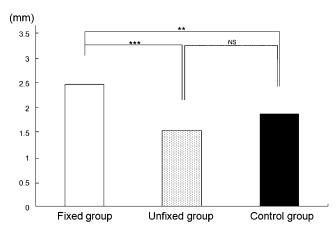


Figure 7. Changes in mandibular length (Cop-Me) throughout the experimental period (T0-4). NS, Not significant; **P < .01; ***P < .001.

dyle immediately after removal of the bite-jumping appliances was less than that occurring naturally in rats in which the appliances were removed early on day 30, although the level of bone formation after the appliances were removed was similar to the level of natural growth in rats in which the appliances were removed late on day 44.7 The reason for this phenomenon was that the emergency type of bone with inherently weak type III collagen matrix as formed during bone development at an early stage would not be stable enough to resist the forces of mastication and normal function.7 In addition, it has been reported that the level of new condylar bone formed by 2 mm-forward mandibular positioning was half that formed by 3.5 mm-forward positioning in rats.6 In the present study, the experimental period until T3 was 21 days, and the inclination of the lower incisors in the unfixed group had already increased at T3 when compared with those in the fixed and control groups. Therefore, the decrease in mandibular growth in the unfixed group

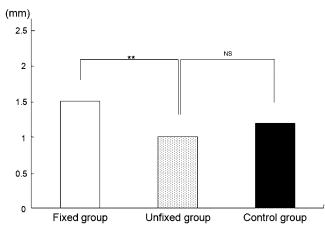


Figure 8. Changes in ramus height (Co-Co') throughout the experimental period (T0-4). NS, Not significant; $^{**}P < .01$.

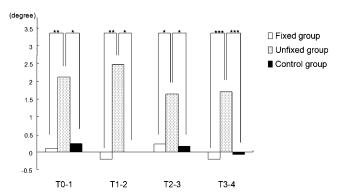


Figure 9. Changes in inclination of the lower incisors in each group. $^*P < .05; ^{**}P < .01; ^{***}P < .001.$

may have been caused by the decrease in mandibular advancement caused by the increasing inclination of the lower incisors, similar to the results obtained previously with early removal of the bite-jumping appliances.⁷

Mandibular growth at T0-4 in the fixed group was significantly greater than in the unfixed and control groups. Therefore, it was suggested that forward mandibular advancement when a fixed functional appliance is used effectively accelerates mandibular growth when labial movement of the lower incisors is prohibited. No significant difference was observed between the unfixed and control groups. The reason for this may be reduced mandibular growth at T2-3 in the unfixed group, as was discussed earlier. It has been reported that the effects of mandibular advancement on condylar growth might be permanent,6 although several other researchers have reported that these effects are not permanent, and that final growth does not change.^{22,23} It was not clear whether the effect of mandibular advancement was permanent or not in the present study, because the experimental period extended only to 8 postnatal weeks, while mandibular growth continued until 6 postnatal months.24 In the fu-

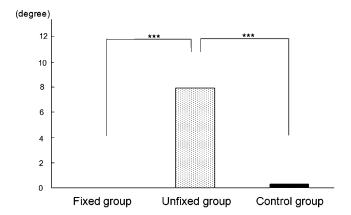


Figure 10. Changes in inclination of the lower incisors throughout the experimental period (T0-4). ***P < .001.

ture, another rat study with an experimental period that includes 6 postnatal months²⁴ should be conducted to determine whether the effects of mandibular advancement are permanent.

CONCLUSION

Mandibular growth in rats was accelerated effectively before and during the pubertal period by mandibular advancement with a fixed functional appliance combined with prohibition of labial movement of the lower incisors.

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