The orthopaedic effects of bone-anchored maxillary protraction in a beagle model

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SUMMARY
BACKGROUND/OBJECTIVES: The purpose of this study was to establish an animal model of bone-anchored maxillary protraction (BAMP) and verify the effects of such treatment in this model.
SUBJECTS/METHODS: Ten total immature (90-day-old) male beagle dogs were used. On Day −20, one miniplate per jaw quadrant was placed and secured with screws. From Day 0 to Day 60, miniplates in the dogs in the intermaxillary traction group (group T, n = 5) were loaded with coil springs. In the control group (group C, n = 5), the miniplates received no force. Every 20 days from Day −20, all dogs were assessed by measuring body weight, taking photographs, and acquiring standardized lateral cephalometric radiographs using a specially designed cephalostat. Cephalometric analyses were performed and the two groups compared. New bone formation was labelled by double-fluorochrome administration with calcein and tetracycline. Animals were sacrificed at Day 60, and bone sections of zygomaticomaxillary sutures were analysed using histomorphometry with fluorescence microscopy. Groups were compared with Mann–Whitney U-tests (P < 0.05).
RESULTS: Cephalometric analysis indicated significant maxillary advancement and retroclination of maxillary incisors in group T, with concomitant significant posterior relocation of the condyles and proclination of the mandibular incisors. In histological analysis, vigorous bone apposition at the zygomaticomaxillary suture was only detected in group T.
LIMITATIONS: Further histological studies would clarify the effects of BAMP on the mandible, especially on temporomandibular articulation.
CONCLUSIONS/IMPLICATIONS: Our results, using this newly developed animal model, support the orthopaedic effects of BAMP.

Introduction

Class III malocclusions are caused by disharmonies of dental components, skeletal components such as the cranial base, maxilla, and mandible, or a combination of both (Jacobson et al., 1974; Guyer et al., 1986; Battagel, 1993). The realization that maxillary deficiency is frequently a component of skeletal class III malocclusion (Guyer et al., 1986) and the development of new treatment modalities for correcting it have prompted an increase in the use of treatments aimed at promoting maxillary growth.

Traditionally, one of most common approaches for class III patients with maxillary deficiency is protraction facemask therapy. The major feature of this treatment is that orthopaedic forces are applied to the maxillary teeth as anchors through which the force is delivered to the maxilla. In prior studies of this treatment, uncertain skeletal effects and unwanted dentoalveolar effects were reported because the forces act indirectly at the maxilla (Baik, 1995; Gallagher et al., 1998; Turley, 2002; Kama et al., 2006).

Therefore, development of a treatment that minimizes the dentoalveolar effects while transmitting the orthopaedic forces to the maxilla would greatly improve this method.

The use of temporary anchorage devices (TADs) in orthodontics has become more common in the last decade. Among a wide variety of applications, TADs have been utilized as anchorage for orthopaedic maxillary protraction (Singer et al., 2000; Enacar et al., 2003; Kircelli and Pektas, 2008). The recently introduced bone-anchored maxillary protraction (BAMP) technique does not require a facemask, instead using class III elastics between miniplates implanted for skeletal anchorage in the upper and lower jaws (De Clerck et al., 2002; De Clerck et al., 2009). With this approach, intermaxillary traction can be applied without interruption (unlike with the facemask, which must be removed at times), and the burden on the patients can be greatly reduced and possibly in turn help them improve compliance. Preliminary studies have indicated that BAMP could achieve successful maxillary protraction even in the
late mixed or permanent dentition phases (age 10–12 years; De Clerck et al., 2009; Heymann et al., 2010). Because of these advantages, BAMP is attracting attention as a potentially effective orthopaedic treatment. Although fairly promising outcomes of BAMP treatment have been reported clinically, based on conventional two-dimensional (2D) cephalometric analyses (Cevidanes et al., 2010; De Clerck et al., 2010) as well as three-dimensional (3D) analyses (Heymann et al., 2010; Nguyen et al., 2011; De Clerck et al., 2012), there is currently no basic information on the biology of this treatment modality. Hence, the aim of this study was to establish an animal model of BAMP using beagle dogs and delineate the mechanism underpinning its effects. It was hypothesized that this animal experimental model could provide biological insights into the application of BAMP therapy as an orthopaedic approach to growing human class III patients.

Materials and methods

Ten immature (90-day-old) male beagle dogs weighing 6.1–7.1 kg (mean body mass: 6.6 kg) were used for this study. The dogs were caged individually and kept under a 14/10 hour light-dark cycle at 21 ± 2 degree C. They were fed a soft dog chow (DSA®, Oriental Yeast Co., Ltd, Tokyo, Japan) once a day in the morning, with water available ad libitum. All dogs were cared for in accordance with the regulations of the Institutional Animal Care and Use Committee at the Tokyo Medical and Dental University under the supervision of licensed veterinarians, and this research was approved by the ethical review board of the Institutional Animal Care and Use Committee at the Tokyo Medical and Dental University (approval number: 0130224A).

Experimental protocol

After arrival at the facility at 90-days old (Day −30), all animals were quarantined for 10 d (Figure 1). At 100-days old (Day −20), titanium miniplates designed for orthodontic anchorage (OrthoAnchor plate®, Dentsply Sankin, Tokyo, Japan) were placed in a manner of one miniplate per jaw quadrant (Figure 2a) and secured with titanium screws (OrthoAnchor screw®, 0.2 mm × 5 mm, Dentsply Sankin, Tokyo, Japan).

The surgical procedures for the implantation of the miniplates were as described previously (Sugawara et al., 2004; Cornelis et al., 2008). Briefly, the animals were anaesthetized with thiopental sodium (Ravonal® 0.3 mg per injection, Tanabe Seiyaku Co. Ltd, Tokyo, Japan) at a dose of 20 mg/kg body weight. Local anaesthesia (2 per cent lidocaine; Dentsply Sankin, Tokyo, Japan) was applied at each miniplate implantation site. A 2 cm incision was made in the buccal mucosa of the maxilla and mandible to allow the reflection of a mucoperiosteal flap. Y-shaped miniplates were fixed on the buccal side of the maxilla with three screws and I-shaped miniplates were fixed on the buccal side of the mandible with two screws. The mucoperiosteal flap was repositioned and the surgical wound sutured with the long arm of the miniplate exposed intra-orally.

After 20 days (Day 0; 120-days old), the dogs were divided into two groups: the intermaxillary traction group (group T; n = 5) and a control group receiving no traction (group C; n = 5). In group T, the miniplates were loaded with stainless steel coil springs tied through the hook on the heads of the implants with 0.4 mm stainless steel ligature wires (Figure 2b). The initial tension of the coil springs was 2.0 N. The intermaxillary traction was continued until Day 60 (180-days old).

Every 20 days from Day −20 to Day 60, all dogs had their body weight measured, and intra-oral photographs and lateral cephalometric radiographs were taken while the dogs were under anaesthesia. The lateral cephalometric radiographs were taken with an X-ray collimator (Collimax Corp., Tokyo, Japan) and a specially designed cephalostat. The head was fixed with ear plugs and a chin fixture. A fixed distance of 83 cm between the source (50 kV, 25 mA, 0.2
second) and the middle of the skull was maintained. The lateral cephalometric radiographs were standardized (1:1.14; Figure 3a). At every stage, the parameters of the two groups (body weight, photographs, and cephalometric analysis) were compared, as described below.

**Cephalometric analysis**

Twelve landmarks used in this study are described in Table 1 and Figure 3b. The Frankfort horizontal (FH) plane was employed as a plane of horizontal reference (x-axis), and a plane constructed perpendicular to the FH plane and passing through the Inion (In) was defined as the vertical reference (y-axis). The horizontal distance between In and the Nasion (N) projected on the x-axis was defined as the reference length (In–N). Nine linear measurements between different pairs of landmarks projected on the x-axis were expressed as percentages of their horizontal distance relative to In–N (Figure 4a). Two angular measurements using the FH plane as a reference were also employed (Figure 4b).

All cephalometric tracings and measurements at the five stages (Days –20, 0, 20, 40, and 60) were made by the same examiner (YI). All cephalometric measurements were repeated after 4 weeks and the method error was calculated with Dahlberg’s formula; method error = \( \sqrt{\frac{\sum d^2}{2n}} \), where \( d \) is the difference between duplicated measurements and \( n \) is the number of double measurements (Dahlberg, 1940; Houston, 1983). The method errors were 0.27–0.64 per cent for linear measurements, and 0.32–0.98 degree for angular measurements.

**Histological analysis**

To assess bone turnover rates, fluorochromes that adsorb to bone mineral were used. Animals received intramuscular injections of two fluorochromes: calcein (20 mg/kg) at Day 45 (165-days old) and tetracycline (30 mg/kg) at Day 55 (175-days old). At Day 60, each dog was sacrificed by injecting an overdose of thiopental sodium intravenously. Then the cranium was recovered for analysis and the miniplates and screws were removed. All specimens were fixed immediately in a 70 per cent ethanol solution. The zygomatico-maxillary sutures were examined in an undecalcified condition. Each tissue block was stained with Villanueva bone stain and embedded in methyl methacrylate. All tissues were cut lengthwise using a circular water-cooled diamond saw in a direction perpendicular to the suture. Fluorescent images were captured with a Zeiss Axiovert 200M microscope fitted with a Zeiss AxioCam MRm camera (Carl Zeiss, Jena, Germany). Histomorphometric parameters were measured at fivefold magnification using Image J 1.44p image-analysis software (National Institute of Health, Bethesda, Maryland, USA). Measurement areas were squares of side length 1.5 \( \times \) 10\(^3\) μm, which included both the zygoma and maxilla, with the suture crossing the centre of the areas. The measured parameters for suture included total bone tissue volume (TV), bone volume (BV), bone surface (BS),
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The static histomorphometric parameters were expressed as a percentage of trabecular bone volume (BV/TV; per cent). The dynamic histomorphometric parameters were mineral apposition rate (MAR, \( \text{MAR} = \frac{L}{10 \text{ days}}; \mu\text{m/day} \)) and bone formation rate (BFR/BS, \( \text{BFR/BS} = \text{MAR} \times \left[ \frac{(dL+S)}{2} \right] / \text{BS} \times 365; \mu\text{m/year} \)). This terminology is in accordance with the American Society for Bone and Mineral Research Committee on Histomorphometric Nomenclature (Parfitt et al., 1987).

Statistical analyses

The body weights and all cephalometric measurements for groups T and C are presented as the median, maximum, and minimum values. Mann–Whitney U-tests were used to detect significant differences between the groups. All statistical tests were performed using SPSS for Windows software (version 19.00; SPSS, Chicago, Illinois, USA). \( P < 0.05 \) was accepted as indicating statistical significance.

Results

There were no significant differences in body weights between the two groups throughout the experimental period (Figure 5). The placement of miniplates was successful in all 10 dogs. No major complications were encountered during the intermaxillary traction procedure.

Oral findings

Representative oral photographs from both groups at each experimental stage are shown in Figure 6. From Day −20 to Day 20, these oral photographs showed no differences between the two groups (Figure 6a and 6b). At Day 40, canine teeth started to erupt and there was a slight tendency to develop class II canine relationships in group T, while class I canine relationships were seen in group C (Figure 6c and 6d). At Day 60, all dogs in group T showed class II canine relationships, whereas all dogs in group C had class I canine relationships (Figure 6e and 6f). In group T, three out of the five dogs displayed an anterior shift of the mandible caused by premature contact between the upper and lower canine teeth (Figure 6e). In group

Table 1 Definitions of cephalometric landmarks used in this study.

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<thead>
<tr>
<th>Skeletal landmarks</th>
<th>Dental landmarks</th>
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<tr>
<td>Or</td>
<td>Orbitale</td>
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<tr>
<td>Po</td>
<td>Porion</td>
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<tr>
<td>In</td>
<td>Inion</td>
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<tr>
<td>N</td>
<td>Nasion</td>
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<td>Pr</td>
<td>Prosthion</td>
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<tr>
<td>St</td>
<td>Staphyilon</td>
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<tr>
<td>Id</td>
<td>Infradentale</td>
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<tr>
<td>Co</td>
<td>Condylion</td>
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| U1 | Upper incisor | Incisal tip of the most anterior maxillary incisor |
| UC | Upper canine | Apex of cusp of the maxillary canine |
| L1 | Lower incisor | Incisal tip of the most anterior mandibular incisor |
| LC | Lower canine | Apex of cusp of the mandibular canine |

Figure 4 Measurements of cephalometric analysis. a: Linear measurements in cephalometric analysis. The horizontal distance between In and the Nasion (N) projected on the x-axis was defined as the reference length (In–N: 100%). Fifteen linear measurements between different pairs of landmarks projected on the x-axis were expressed as percentages of their horizontal distance relative to In–N as follows; 1: In–Or, 2: In–Pr, 3: In–St, 4: In–Id, 5: In–Co, 6: In–U1, 7: In–UC, 8: In–L1, 9: In–LC. For definition of each landmark, refer to Table 1. b: Angular measurements in cephalometric analysis. Angles between various anatomical planes and the Frankfort horizontal (FH) reference plane were measured. Numbers show the position of the angle formed between the FH plane and U1 axis (1) and L1 axis (2, FMIA).

single-labelled surface (sLS), double-labelled surface (dLS), and the width between different fluorochrome labels (L,Th). The static histomorphometric parameters were expressed as a percentage of trabecular bone volume (BV/TV; per cent). The
C, one animal had an anterior crossbite (Figure 6f) and another had an edge-to-edge bite, whereas in group T, none of the animals had either a crossbite or an edge-to-edge bite (Figure 6e).

Cephalometric analysis

The cephalometric analyses of both groups at each stage are shown in Tables 2 and 3. At Day −20 and Day 0, there were no significant differences between the groups in any of the cephalometric parameters measured.

In our maxillary data, only one linear measurement (In–Or) from group T was significantly greater than the equivalent measurement in group C ($P < 0.05$) at Day 20. This indicates a significant advancement only in the orbital ridge at this time point. At Day 40, six linear measurements from group T were significantly greater than their equivalents in group C (In–Pr, In–Or, In–U1, In–UC; $P < 0.05$), indicating significant maxillary advancements in the alveolar bone and orbital ridge. In contrast, the In–St measurement in group T at Day 40 was not significantly different from group C, but this situation had changed by Day 60, at which point this parameter was significantly larger in group T ($P < 0.05$). Furthermore, one angular measurement (U1–FH; $P < 0.05$) in group T was significantly smaller than the equivalent angle in group C at Day 60. These data illustrate a significant advancement in the pterygomaxillary fissure and retroclination of the maxillary incisors. There was no significant difference in UC at Day 60, despite being significantly different at Day 40.

In our mandibular data, there were no significant differences between the two groups in any of the cephalometric parameters measured between the two groups at Day 20. At Day 40 and 60, two linear measurements from group T were significantly greater than those in group C (In–L1 and In–LC; $P < 0.05$), and at Day 60, one linear measurement (In–Co) and one angular measurement (FMIA) from group T were significantly smaller ($P < 0.05$) than in group C. These data describe a positional relocation of the condyles and proclination of the mandibular incisors.

Histological analysis

Representative fluorescence images of the zygomaticomaxillary sutures are shown in Figure 7. Fluorescent tetracycline/calcein labelling can be seen in both groups. In group T, the distances between the two fluorescent labels were evident and greater than those seen in group C. Furthermore, the shape of the ossification front of the suture in group T was more complex and corrugated, while that in group C looked rather smooth. The histomorphometric analysis of both groups is shown in Figure 8. In the static histomorphometry, $BV/TV$ in group T was significantly smaller than in group C ($P < 0.01$). The dynamic histomorphometry of the suture demonstrated that MAR and BFR/BS in group T were significantly larger than in group C ($P < 0.01$). These data indicate vigorous bone apposition and dilation of the zygomaticomaxillary suture took place at in group T, but not in group C.

Discussion

This study is the first investigation into the orthopaedic effects of BAMP using an animal model. Specific features
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<th>Table 2</th>
<th>Cephalometric measurements from Day −20 to Day 20.</th>
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<tr>
<td></td>
<td>Day −20</td>
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<tr>
<td></td>
<td>Group T</td>
</tr>
<tr>
<td>In–Or (%)</td>
<td>Median 91.7</td>
</tr>
<tr>
<td>In–Pr (%)</td>
<td>Median 159.1</td>
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<tr>
<td>In–St (%)</td>
<td>Median 66.7</td>
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<tr>
<td>In–Id (%)</td>
<td>Median 145.1</td>
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<tr>
<td>In–Co (%)</td>
<td>Median 36.9</td>
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<tr>
<td>In–U1 (%)</td>
<td>Median 149.4</td>
</tr>
<tr>
<td>U1–FH (°)</td>
<td>Median 124.2</td>
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<tr>
<td>FMIA (°)</td>
<td>Median 31.7</td>
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NS, not significant. *, P < 0.05.
of this study were the establishment of an experimental model of BAMP and the verification of the treatment effects in the beagle dogs in the growing stage.

Beagle dogs are extensively used for animal studies because of their docility and ability to use the same intra-oral devices as humans, and many previous studies on TADs have been reported by using beagles (Roberts et al., 1989; Vande Vannet et al., 2007; Deguchi et al., 2011). Aside from these advantages, the growing period of beagles is comparatively short, making it especially suitable for studying an orthopaedic treatment intended for application during the growing stage.

In this study, the strain and age of beagle dogs were matched in both groups, and there were no significant differences in body weight between the two groups throughout the experimental period. The only difference between the groups was the application (or not) of intermaxillary traction, and the experimental model thus examines purely the traction effect in BAMP.

For the analysis of craniofacial morphology in this study, firstly, the lateral cephalometric radiographs were taken using standardization methods as described in previous studies (Losken et al., 1994; Wang et al., 2006; Kim et al., 2008), which included strict control of the voltage, current, and exposure time. Secondly, the landmarks were chosen according to definitions made in previous cephalometric studies (Losken et al., 1994; Stalmans et al., 2003; Wang et al., 2006) and morphometric studies using animal models (Onar, 1999). Finally, the linear and angular measurements of cephalometric analysis were determined by reference to past human studies (Tweed, 1946; Downs, 1948; Coben, 1955; Ricketts, 1960; Cevidanides et al., 2010; De Clerck et al., 2010). In the analysis of Coben, the horizontal distance between the Basion (Ba) and N was defined as the reference length (Coben, 1955). However, in this study, we used the horizontal distance between In and N as the reference length because 1. it is comparatively easy to trace In rather than Ba; 2. In–N has been shown to be an important parameter in previous morphometric studies (Onar, 1999); and 3. using In–N as a reference length permits normalization of individual differences in head size.

The experimental conditions for intermaxillary traction were derived from previous reports on BAMP (De Clerck et al., 2009; Cevidanides et al., 2010; De Clerck et al., 2010; Heymann et al., 2010; Nguyen et al., 2011; De Clerck et al., 2012), in which the initial magnitude of tensile strength of the intermaxillary traction ranged from 1.5–2.5 N (De Clerck et al., 2009; Cevidanides et al., 2010; De Clerck et al., 2010; Heymann et al., 2010; Nguyen et al., 2011; De Clerck et al., 2012). We thus chose 1.96 N as the initial tension magnitude. The sites for miniplate placement were determined by the criteria of anatomical absence of potentially complicating blood vessels and nerves, and the vector of intermaxillary traction force ideally passing through the temporomandibular joint. The period of intermaxillary traction was set at 60 days, to match the growth spurts of beagle dogs.
In our assessment of the maxillary changes, comparison of the cephalometric data and oral findings from group T with those in group C showed significant advancement of only the orbital ridge at Day 20. This early change might have occurred in the areas that were closest to the miniplates. At Day 40, there were significant maxillary advancements in the alveolar bone and orbital ridge, and at Day 60, additional significant maxillary advancements were seen in the pterygomaxillary fissure. These data corroborate findings reported in human patients treated with BAMP (De Clerck et al., 2009; Cevidanes et al., 2010; De Clerck et al., 2010; Heymann et al., 2010; Nguyen et al., 2011). The effect of maxillary advancement was also detected histologically. Vigorous bone apposition and dilation of the zygomaticomaxillary suture suggested that the orthopaedic force acted directly on the suture. There was no significant advancement in UC at Day 60, despite the groups having significantly different UC measurements at Day 40, which could be explained by a premature contact between the upper and lower canines resulting in retroclination of the maxillary canines between Day 40 and 60. Indeed, significant retroclination of the maxillary incisors was detected, suggesting that the maxillary bone moved bodily forward as a result of the intermaxillary traction and this movement was compensated for by repositioning of the maxillary incisors.

In our assessment of the mandibular changes by a comparison of the cephalometric data and an examination of oral cavity between the two groups, significant posterior relocation of the condyles and proclination of the mandibular incisors were observed in group T at Day 60, in accordance with previous clinical studies (Heymann et al., 2010; De Clerck et al., 2010; De Clerck et al., 2012). Proclination of the mandibular incisors is believed to be the result of dental compensation for maxillary advancement. Significant restraining effects on mandibular growth both at the B-point and at the Pg were reported in previous studies of BAMP for class III patients, (De Clerck et al., 2010; De Clerck et al., 2012). In this study, however, we saw no significant advancement of Id, but this does not rule out a restraining effect on mandibular growth in the anterior region of the mandible because the
position of Id might be affected by the position and inclination of the mandibular incisors.

Our cephalometric findings largely coincide with the data of clinical studies of BAMP treatment for human class III patients and support the application of this orthopaedic approach. In addition, we have verified the effect of BAMP on the zygomaticomaxillary suture throughhistological evaluation. However, further histological studies would be necessary to clarify the effect of BAMP on the mandible, especially the mandibular condyle and the glenoid fossa with which it articulates. Furthermore, the BAMP protocol could be further refined by optimizing conditions such as the traction magnitude, vectors, and duration.

Conclusions
We have established an animal model of BAMP and have verified the treatment effects of BAMP. Our results may support the clinical application of BAMP as an orthopaedic approach for growing class III patients.

Funding

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