Original Article

Effects of Bisphosphonate on the Remodeling of Rat Sagittal Suture After Rapid Expansion

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Abstract: The purpose of this study was to investigate the effects of bisphosphonate (BP), an inhibitor of bone resorption, on the remodeling of the rat sagittal suture after rapid expansion. Wistar strain male rats were divided into 4 groups and subjected to rapid mechanical expansion with an initial force of 60 g for 3 days. Immediately after the sutural expansion, the appliance was removed in 2 of the groups. In the S group, half of the animals were injected with saline solution daily for 3 days and the other half were injected daily for 7 days prior to sacrifice. In the B group, half of the animals were injected with bisphosphonate daily for 3 days and the other half were injected daily for 7 days prior to sacrifice. The remaining 2 groups underwent 7 days of mechanical retention before the appliance was removed. In the SR group, half of the animals were injected with saline solution daily for 3 days and the other half were injected daily for 7 days prior to sacrifice. In the BR group, half of the animals were injected with bisphosphonate solution daily for 3 days and the other half were injected daily for 7 days prior to sacrifice. The calvariae were fixed in 4% neutral formalin, decalcified, and embedded in paraffin. The amount of relapse and the relevant bone remodeling were evaluated in terms of a relapse ratio and the number of osteoclasts. The relapse ratio was 54.11% in the S group and 32.53% in the B group 7 days after the injection. This ratio was 25.13% in the SR group and 9.60% in the BR group. The number of osteoclasts 3 days after injection was 15.47 in the S group, which was significantly greater than the 5.26 present in the B group. This number was 7.08 in the SR group, which was significantly greater than the 1.83 in the BR group. These results demonstrate that the injection of BP after rapid expansion, if combined with mechanical retention, may produce more secure retention by inhibiting bone resorption, indicating a possibility of employing a pharmaceutical aid to decrease the skeletal relapse after mechanotherapy in clinical orthodontics. (*Angle Orthod* 2001;71:265–273.)

Key Words: Bisphosphonate; Rapid expansion; Rat sagittal suture; Retention; TRAP-positive cell

INTRODUCTION

In clinical orthodontics, the relapse of a tooth previously moved during treatment is regarded as a cumbersome problem. In order to maintain the treatment outcomes with long-term stability, relapse is currently prevented by mechanical retention with various appliances. Any relapse that does occur is considered a result of the resorption of the new bone formed in the tension area where the previous tooth movement occurred. Based on these considerations, it is of great significance to explore some other effective approaches to the inhibition of bone resorption as a potential key determinant in preventing orthodontic relapse.

Bisphosphonate (BP) is a compound characterized by a P-C-P bond and is related to pyrophosphonate. BP has an in vitro inhibitory effect on calcium phosphate formation[1,2] and hydroxyapatite dissolution.[3,4] A high affinity to hydroxyapatite crystals is recognized as the mechanism of inhibiting both in vitro calcium dissolution and in vivo bone resorption.

A dose-dependent inhibitory effect of BP on in vivo soft tissue calcification has been reported.[5] BP also inhibits both accretion of mineral into bone[6] and bone resorption[3,4] when given subcutaneously or orally.[7] BP is an well-established therapeutic inhibitor of bone resorption and has been used for the treatment of Paget's disease, malignant hypercalcemia, and osteoporosis.[8,9]

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The effects of BP on bone metabolism may result from a high binding affinity to hydroxyapatite, which causes the further accumulation on bone mineral surfaces. BP inhibits bone resorption by injuring or giving cytotoxins to mature osteoclasts. Moreover, bisphosphonate–bone complex also inhibits the direct excavation of resorption areas by mature osteoclasts.[10–12] Igarashi et al[13] reported that 4-amino-1-hydroxybutylidene-1,1-bisphosphonate (AHBuBP) reduced orthodontic tooth movement or relapse in rats when administered systemically or topically. Furthermore, Adachi and associates[14] showed that the topical administration of risedronate inhibited the relapse of tooth movement and was dose dependent. These results suggest that BP can prevent tooth movement and relapse of the moved teeth.

Sutural expansion with mechanical forces is accomplished by a stretching of the collagenous fibers accompanied by new bone formation with associated mitotic figures. After the desired expansion is complete, the suture undergoes remodeling, which includes bone resorption and formation and fiber rearrangement. This continues until the architectural environment achieves equilibrium.

In clinical orthodontics, various retainers are used after therapeutic expansion to hold the positions of the teeth and to allow the periodontal tissues to reorganize themselves. Many previous studies have reported on the association of mechanical expansion with suture remodeling;[15–17] however, the effects of mechanical retention and the association of bone remodeling and relapse after rapid expansion remains unclear.

Based on the facts previously described, it is hypothesized that BP prevents skeletal relapse after rapid expansion of the suture. In order to eliminate the influence of occlusal forces or mastication, the present study was designed to examine the effect of expansion forces directly on the remodeling of the rat sagittal suture and the subsequent relapse. The purpose of this study was to explore the possibility of pharmaceutically controlling or decreasing skeletal relapse.

MATERIALS AND METHODS

Experimental animals

Forty-four 7-week-old male Wistar strain rats with a mean weight of 223.9 ± 11.1 g were divided into 4 experimental groups of 5 to 6 animals each. The animals were treated under ethical regulations as defined by the Ethics Committee, Hiroshima University Faculty of Dentistry. They were fed an ordinary solid diet containing 1.18 g Ca, 1.03 g P, and 250 IU vitamin D3 per 100 g (Japan Clea, Tokyo, Japan) with water ad libitum and were kept in cages at 25°C under alternate 12-hour periods of light and dark conditions. Body weight was measured every day during the entire experimental period.

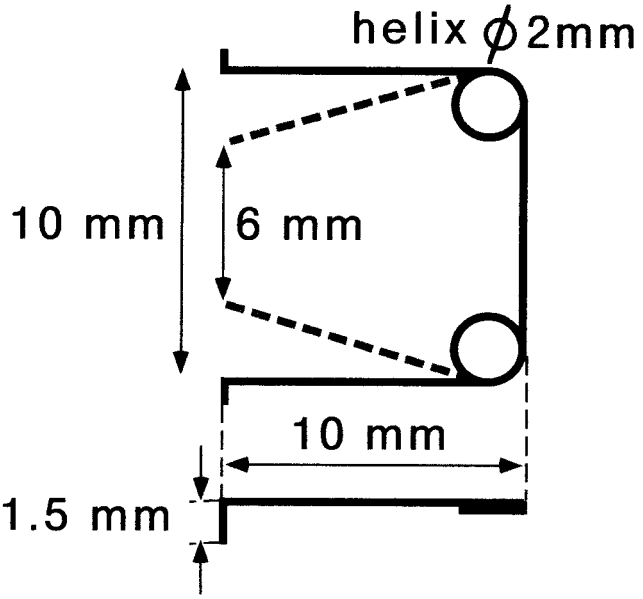

FIGURE 1. Schematic illustration of expansion appliance. An expansion appliance was made of 0.5-mm chromium-cobalt alloy wire with 2 helices; solid lines denote the view without activation and interrupted lines denote the view with activation.

Mechanical expansion of the sagittal suture

Suture expansion was carried out for 3 days for all animals using an expansion appliance made of 0.5-mm chromium-cobalt alloy wire (Sankin, Tokyo, Japan) with 2 helices. Under general anesthesia with sodium pentobarbital (Nembutal, Dinabot, Osaka, Japan), a midsagittal incision was made anteroposteriorly through the scalp to expose the sagittal suture. Two holes were symmetrically placed in the parietal bones on opposite sides of the suture with a hole-to-hole distance of 6 mm. The expansion appliance was calibrated in advance to exert an initial expansion force of 60 g and was placed into the holes. Finally, the scalp was sutured shut with swabbing isozin (Figures 1 and 2).

Immediately after the sutural expansion period, the appliance was removed in 2 of the groups. Physiological saline solution (5 mg/kg, 0.9% NaCl) was injected subcutaneously into the animals in 1 group (the S group) and bisphosphonate (etidronate) dissolved in physiological saline (5 mg/kg) was injected subcutaneously into the second group (the B group). In both groups, half of the animals were injected daily for 3 days and the other half were injected daily for 7 days with their respective solutions prior to sacrifice.

The remaining 2 groups underwent 7 days of mechanical retention using the expansion appliance as a retaining device whose loops were fixed with acrylic resin. At the conclusion of the 7-day retention period, the appliance was removed. Physiological saline was injected subcutaneously into this third group (the SR group) and BP was injected into the fourth group (the BR group) (Table 1). Again, in both groups, half of the animals were injected daily for 3

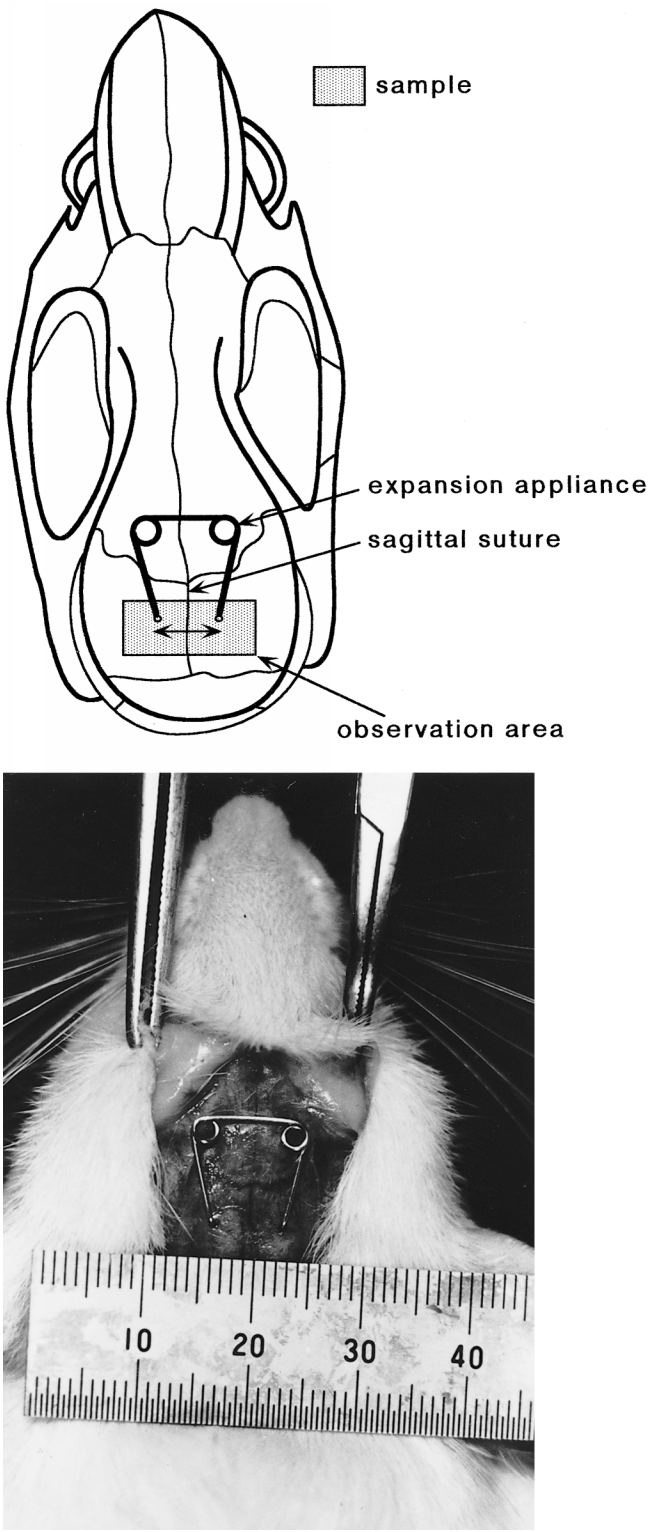

FIGURE 2. (A) Schematic illustration and (B) actual view (right) of expansion appliance placed on rat's sagittal suture. Two holes were formed symmetrically on the parietal bones facing the suture with a hole-to-hole distance of 6 mm. The expansion appliance was calibrated in advance to exert an initial expansion force of 60 g and placed into the holes.

TABLE 1. Number of Animals in Experimental Groups

	3-Day-Injection	7-Day-Injection
Nonretention group		
B group	6	6
S group	6	6
Retention group		
BR group	5	5
SR group	5	5

days and the other half were injected daily for 7 days with their respective solutions prior to sacrifice.

Amount of sutural expansion and the relapse ratio

The sagittal suture, including the holes on the parietal bones, was photographed before insertion of the appliance, immediately after expansion, at the removal of appliance, at the beginning of retention, and at the end of the experiment. The pictures were entered into a personal computer with an image scanner (Nikon Coolscan, Tokyo, Japan). The distance between the holes was measured using image-analyzing software (NIH Image version 1.59, National Institutes of Health, Bethesda, Md) for each of the experimental stages. The relapse ratio or the rate of decrease in the distance was calculated according to the following equation:

$$(A - B)/(A - C) \times 100,$$

where A = the distance at the removal of expansion appliance for nonretention S and B groups or the distance after a 7-day retention for the SR and BR groups, B = the distance after 3- or 7-day observation, and C = the distance at the beginning of the experiment.

Histological observations

At the end of each experimental period, the animals were sacrificed under general anesthesia with sodium pentobarbital. Half of each group was sacrificed after 3 days and the other half after 7 days. The heads of all animals were dissected and fixed in 4% neutral buffered formalin for 2 days. The specimens were rinsed with phosphoric acid buffer solution, decalcified in 14% EDTA for 4 weeks, and washed again by the same buffer solution. After dehydration in ethanol, the parietal bones, including the sagittal suture, were removed carefully and embedded in paraffin. The specimens were cut into frontal sections of 4.5-μm thickness.

For the histological and histochemical examinations, the sections were stained with hematoxylin and eosin (HE) as well as tartrate-resistant acid phosphatase (TRAP). The histochemical staining for TRAP was made according to the methods described by Cole and Walters[18] and Farrell et al[19]

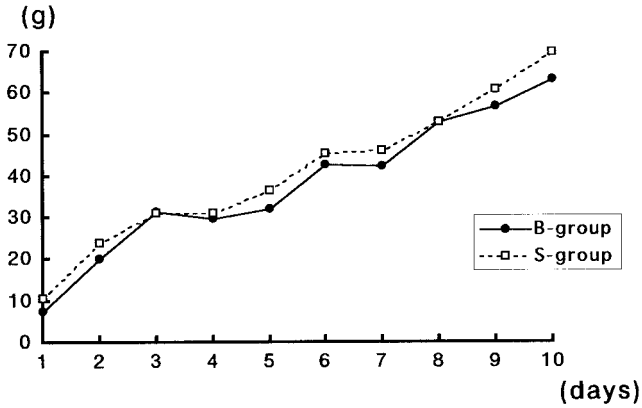

FIGURE 3. Changes in body weight in the B and S groups. Significant differences in the changes of body weight were not found between the B and S groups during the experiment.

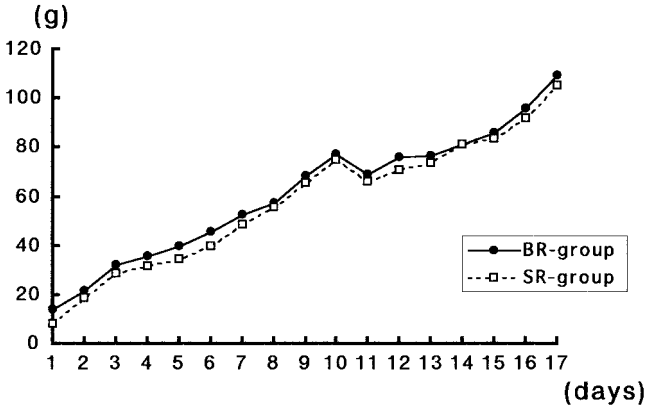

FIGURE 4. Changes in body weight in the BR and SR groups. No significant differences in the body weight changes were found between the BR and SR groups during the experimental period.

and was observed with a light microscope (BH-2-RFCA, Olympus, Tokyo, Japan).

The number of TRAP-positive multinuclear cells existing on the bone surfaces at the sutural interface was counted. The values for 4 sections were averaged for each rat and used to calculate the mean and standard deviation for each experimental group.

Statistical treatment

In this study, all the data were first subjected to an *F*-test to examine the differences in the variances between 2 groups. Fisher's test for the protected least significant difference was then used to examine the mean differences between the 2 groups.

These analyses were performed with the statistical program StatView 4.11J (Abacus Concepts Inc, Berkeley, Calif) on a personal computer (Power Macintosh 1400c, Apple Computer Inc, Cupertino, Calif).

RESULTS

Changes in body weight

Changes in body weight are shown in Figures 3 and 4. No significant difference was found in the daily changes of body weight between the S and B groups during the experiment. Similarly, no significant differences in the body weight changes were found between the SR and BR groups during the entire experimental period. These findings confirmed that this series of experiments on general growth of the rats, including surgical invasion and BP injection, had a negligible influence in terms of changes in the body weight.

The amount of sutural expansion

Figures 5 and 6 show changes in the distance between the 2 holes, or the amount of sutural separation. After the

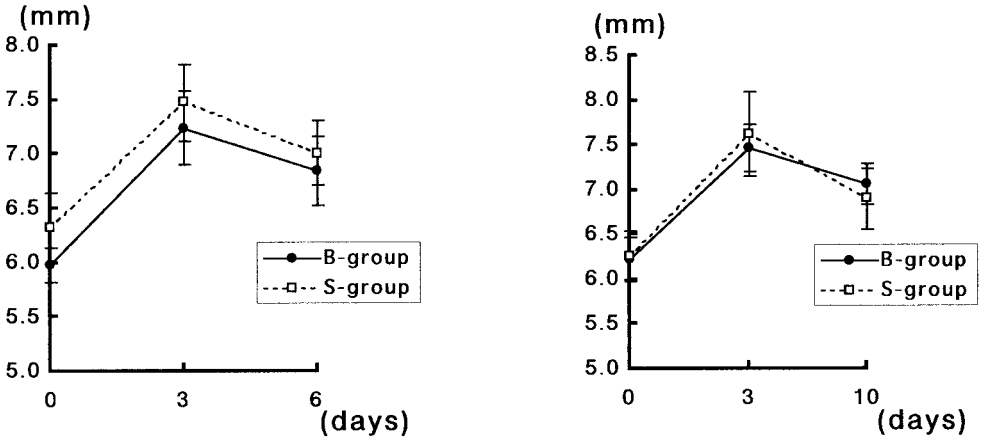

FIGURE 5. Changes in the distance between 2 holes in the B and S groups. After a 3-day expansion, the sagittal suture was separated by about 1.26 mm. The distances decreased gradually after removal of the appliances in the S and B groups. Furthermore, the rate of decrease was more substantial in the S group than in the B group.

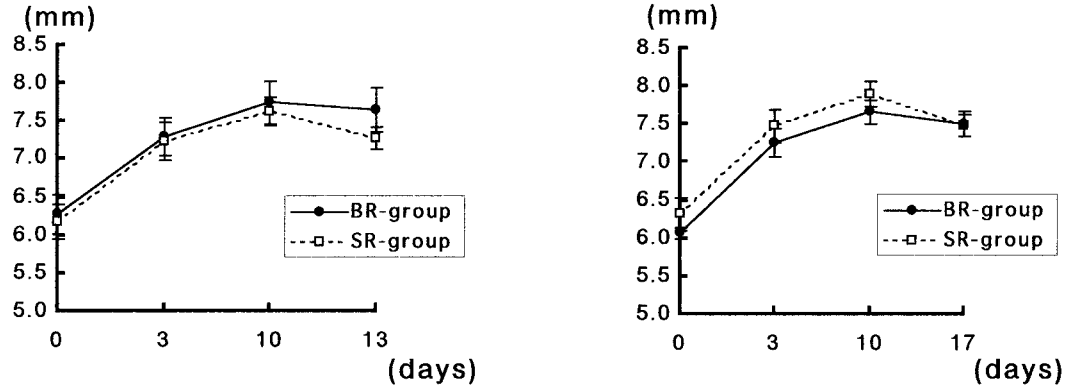

FIGURE 6. Changes in the distance between 2 holes in the BR and SR groups. In the SR and BR groups, the distances slightly increased during retention for 7 days. After removal of the retainer, however, the distances gradually decreased. The rate of decrease was significantly greater in the SR group than in the BR group.

TABLE 2. Relapse Ratios in Four Experimental Groups and the Differences

Duration of Injection, days	Experimental Group, mean ± SD				Difference Between Groups[a]		
	B	S	BR	SR	B vs S	BR vs SR	B vs BR
3	31.39 ± 8.84	40.29 ± 5.74	6.86 ± 3.19	23.80 ± 3.66	*	**	**
7	32.53 ± 3.48	54.11 ± 13.70	9.60 ± 5.72	25.13 ± 6.43	**	**	**

[a] *, significantly different at 95% level of confidence; **, significantly different at 99% level of confidence.

3-day expansion, the sagittal suture was separated by about 1.26 mm.

The distance between the holes decreased gradually after removal of the appliances in the S and B groups. Furthermore, the rate of decrease was more substantial in the S group than in the B group (Figure 5).

In the SR and BR groups, the distances increased slightly during the 7-day retention period. After removal of the retainer, however, the distances gradually decreased. The rate of decrease was significantly greater in the SR group than in the BR group (Figure 6).

Relapse ratio

Changes in the relapse ratio are shown in Table 2. The relapse ratio in the B group was significantly less than in the S group after 3 days of injections. After 7 days of injection, the ratio in the B group was 32%, which was also significantly smaller than the 54% ratio in the S group (Table 2).

In the BR group, the relapse ratio was significantly smaller than in the SR group after both a 3 and 7-day injection period. A similar finding was observed after a 7-day injection period, where the BR group relapse ratio was 9%, which was significantly smaller than the 25% relapse ratio in the SR group (Table 2).

Histological findings

Figures 7 and 8 show photomicrographs of the sagittal suture subjected to mechanical separation. TRAP activity was greater, with more numerous multinuclear cells on the sutural bony surfaces in the S and SR groups than in the B and BR groups (Figures 7 and 8). Meanwhile, fewer TRAP-positive cells were observed apart from the sutural borders, indicating a lower TRAP activity in the B and BR groups.

No prominent histological differences were found between the S and B groups, excluding an appearance of TRAP-positive cells (Figure 7). The sutural bony surfaces showed a smoother curve in the SR and BR groups after 7 days of injection compared with those after a 3-day injection period (Figure 8). In addition, a single layer of flattened osteoblasts exhibited a more uniform and better arranged alignment in the BR group after 7 days of injection compared with the remaining groups. This finding indicates more progress in tissue restructuring in the BR group, which may be due to the function of BP.

The number of TRAP-positive cells

Table 3 shows the number of TRAP-positive cells. After 3 days of injections, there were 5.3 TRAP-positive cells in the B group, which was significantly less than the 15.5 TRAP-positive cells in the S group. These numbers decreased gradually and became significantly less in the 7-day injection groups compared with the 3-day injection groups.

Meanwhile, the number of TRAP-positive cells was 1.8 in the BR group, which was significantly less than the 7.1 TRAP-positive cells in the SR group. Furthermore, the val-

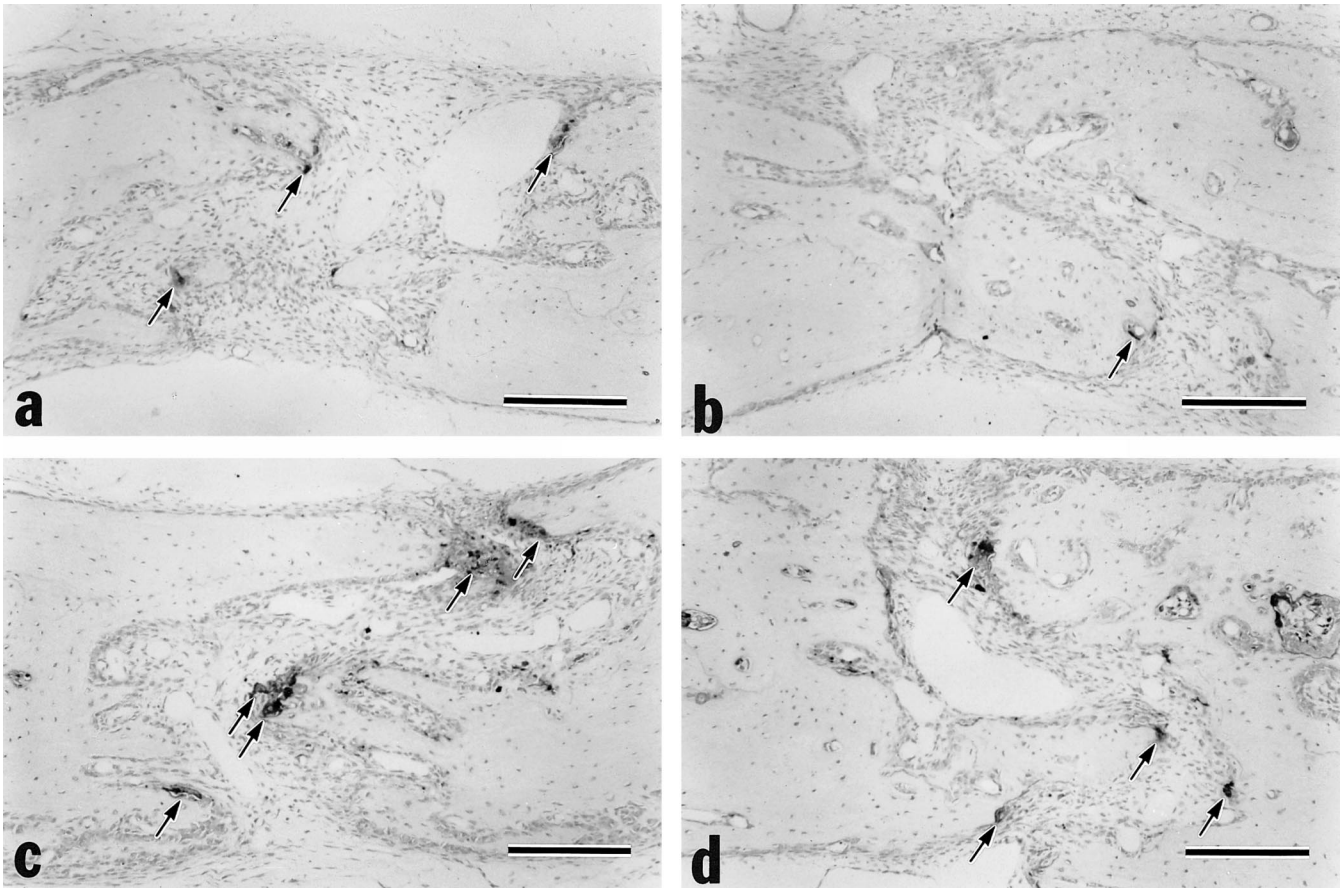

FIGURE 7. Appearance of TRAP-positive cells in the sutural space of the S and B groups. (a) The B group after 3-day injection of bisphosphonate; (b) the B group after 7-day injection of bisphosphonate; (c) the S group after 3-day injection of saline solution; (d) the S group after 7-day injection of saline solution. TRAP activity counterstained with hematoxylin. Arrows indicate TRAP-positive cells. Bars = 200 μm.

ue was significantly less in the SR and BR groups than in the corresponding S and B groups (Table 3).

DISCUSSION

A previous study reported that bisphosphonate (BP) prevented orthodontic tooth movement and relapse.[13] The reason for an initial relapse of moved teeth within 24 hours is that the deformed periodontal ligament returns to the original form, releasing the internal strain energy.[20,21] The relapse of sutural expansion is considered to be due to a similar mechanism. It is known that the rate of relapse gradually decreases as the retention lasts longer.[22] Thus, the effects of mechanical retention on relapse have been shown. However, the effects of BP on sutural modification after rapid expansion have not been examined.

In the present study, the relapse ratio was significantly smaller in the SR group than in the S group. This result demonstrates that mechanical retention is the most important factor for the prevention of relapse. The relapse ratio was also significantly smaller in the B group than in the S group. This finding indicates that the degree of relapse after rapid expansion of the sagittal suture is reduced significantly by the injection of BP and the effects are dependent on the duration of injection. In addition, the relapse ratio was smaller in the BR group than in the SR group. Thus, it is confirmed that injection of BP combined with mechanical retention produces more secure retention for the mechanically separated suture.

Igarashi et al[13] and Adachi et al[14] reported that both the amount of orthodontic tooth movement and the degree of relapse were reduced by the topical administrations of 4-amino-1-hydroxybutylidene-1,1-bisphosphonate (AHBuBP) and risedronate, respectively. In this study, relapse of mechanical sutural expansion was controlled or reduced by injection of BP, etidronate. Although the dose and duration of injection were the same, the degree of relapse was significantly less in the BR group than in the B group. This may be because the amount of new bone formation was greater in the BR group than in the B group as a result of the mechanical retention period. It is speculated that the new bone may play a stronger resistance against the resorption leading to bony relapse. High affinity of BP to hydroxyapatite crystals may also be pertinent to reduce the bone resorption essential for the relapse.

Histological examination in this study revealed that the

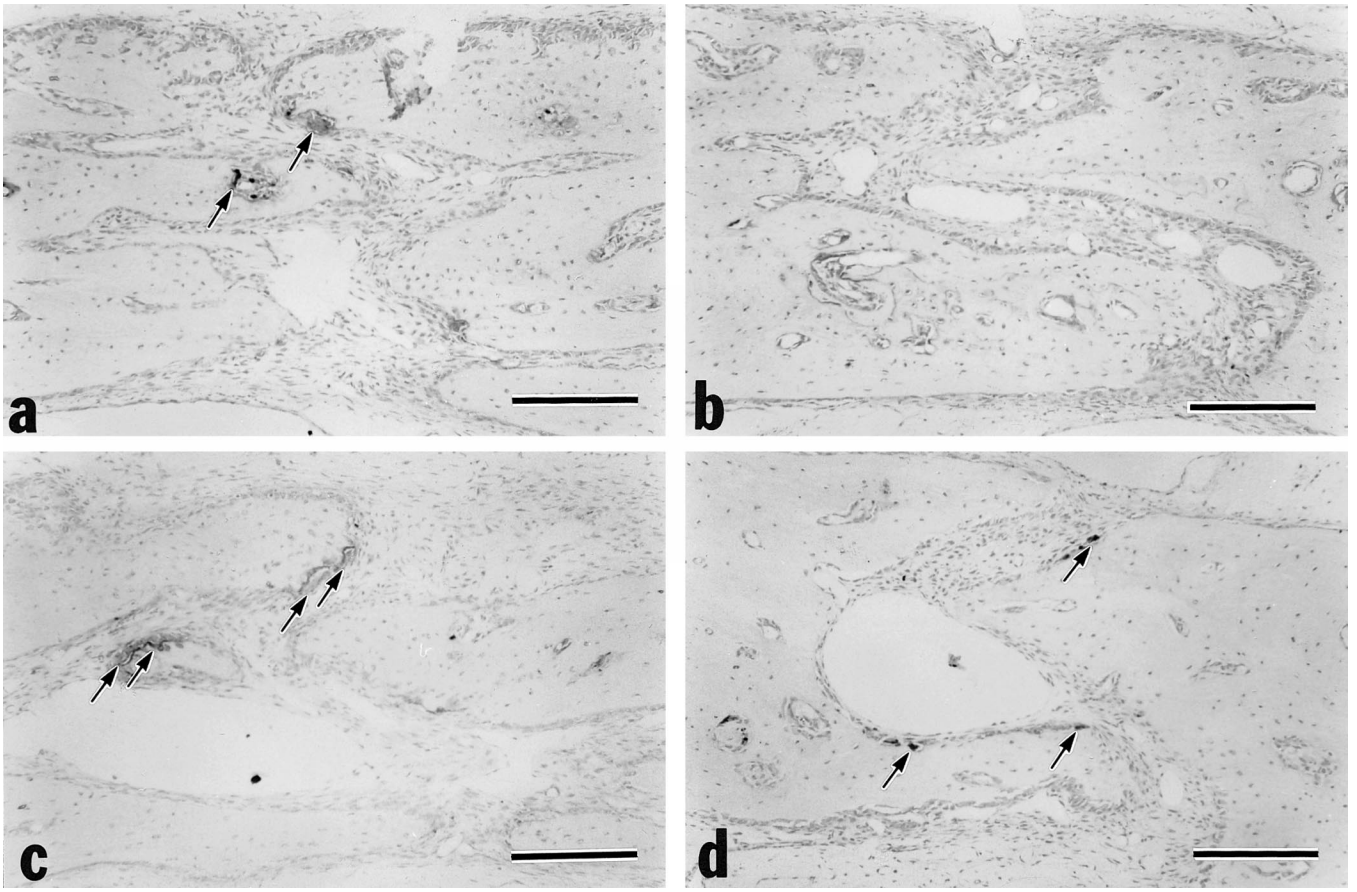

FIGURE 8. Appearance of TRAP-positive cells in the sutural space of the SR and BR groups. (a) The BR group after 3-day injection of bisphosphonate; (b) the BR group after 7-day injection of bisphosphonate; (c) the SR group after 3-day injection of saline solution; (d) the SR group after 7-day injection of saline solution. TRAP activity counterstained with hematoxylin. Arrows indicate TRAP-positive cells. Bars = 200 μm.

TABLE 3. Number of TRAP-positive Cells (per section) in Four Experimental Groups and the Differences

Duration of Injection, days	Experimental Group, mean ± SD				Difference Between Groups[a]			
	B	S	BR	SR	B vs S	BR vs SR	B vs BR	S vs SR
3	5.26 ± 1.09	15.47 ± 4.06	1.83 ± 1.21	7.08 ± 1.66	**	**	*	**
7	1.85 ± 0.83	10.56 ± 3.40	2.37 ± 2.57	6.60 ± 1.65	**	**	NS	**

[a] NS, not significantly different; *, significantly different at 95% level of confidence; **, significantly different at 99% level of confidence.

number of multinuclear giant cells decreased in the BP injection group compared with the saline injection group. Warita et al[23] have recently reported that topical application of 1-hydroxyethylidene-1,1-bisphosphonate (HEBP) inhibited experimental tooth movement in rats and decreased the number of multinuclear giant cells on the pressure side. Evans et al[24] indicated that the number of osteoclasts decreased after the injection of BP, ethane-1-hydroxy 1,1-diphosphonate (EHDP) of 2 mg/kg/d given to male rats for 140 days. They also revealed that metaphyseal bone areas increased with a decrease in the number of osteoclasts, although diaphyseal bone area did not vary. Therefore, it is considered that biologic responses of osteoclasts to BP may exhibit a site-specific difference.

Mühlbauer et al[25] reported that the number of osteoclasts increased in animals given 4 mg/kg/d BP, indicating that the inhibition of bone resorption was not due to an inhibition of osteoclast recruitment. They also indicated that the number of osteoclasts decreased at the high dosage. Marshall et al[26] reported that the number of osteoclasts increased with relatively low doses of BP. Meanwhile, many studies have demonstrated that BPs inhibited both differentiation of osteoclasts from their precursors and the recruitment.[10,11,27–31]

In this study, BP of 5 mg/kg/d was injected into rats for 1 week. The dose seemed relatively low; however, the number of osteoclasts decreased progressively during the experimental period. Although few osteoclasts appeared in the sagittal suture without mechanical separation, many osteoclasts were induced by the relapse of sutural expansion in this experiment. These results support the finding that inhibition of bone resorption by BPs is due to a decrease in the number of osteoclasts, based on the assumption that bone resorption depends on the formation of new osteoclasts.[28,30,32] Therefore, originally existing osteoclasts seem to be related to the formation of new osteoclasts and obviously appeared in the group with more substantial relapse. It may thus be assumed that the number of osteoclasts decreased progressively during the experimental period due to the inhibition of their recruitment and differentiation from their precursors.

The histological examinations of the BP-injected group showed that some multinuclear giant cells were located apart from the sutural bony surfaces and that the TRAP activity was reduced compared with the saline injection group. Moreover, defects in the ruffled border were found in osteoclasts at the sutural interface in the BP injection group. Kameda et al[12] and Hughes et al[11] suggested that apoptosis of osteoclasts may be a major role of BPs in reducing the induction and activity of osteoclasts. Igarashi et al[13] reported that direct bone resorption by osteoclasts was observed on the alveolar bone surface in the control animals but not in the AHBuBP-treated animals. A lesser number of multinuclear cells appeared on the alveolar bone surface in the AHBuBP-treated animals whereas some were located apart from the bone surface and exhibited rounded shapes, loss of polarity, and pycnosis.[13] In the present study, changes in the microstructural level of osteoclasts were not shown; however, it would be a reasonable assumption that injection of BPs could affect the structure and function of osteoclasts and consequently cause the apoptosis.

CONCLUSIONS

The effects of bisphosphonates on the remodeling of rat sagittal suture after rapid expansion were examined histochemically with a special reference to the degree of relapse and the changes in the number of TRAP-positive osteoclasts.

A relapse ratio of 54% was found in the S group and 32% in the B group 7 days after the injection. The relapse ratio, meanwhile, was 25% in the SR group and 10% in the BR group. Significant differences were found between the S and B groups and between the SR and BR groups.

The number of osteoclasts in the B group 3 days after injection (5.3) was significantly less than the number of osteoclasts in the S group (15.5). This number was 1.8 in the BR group, which was significantly less than the 7.1 in the SR group.

These results have demonstrated that the injection of BP after mechanical expansion of the suture may produce more secure retention if combined with mechanical retention. This suggests a possibility of pharmaceutically assisted retention to help hold the outcome of sutural mechanotherapy in clinical orthodontics.

ACKNOWLEDGMENTS

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