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Effects of fluoride on osteoclastic bone resorption during experimentally moved rat molars

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Orthodontic tooth movement requires remodelling of periodontal tissues, especially alveolar bone. Fluoride is known to be a potent inhibitor of osteoclastic bone resorption.

The purpose of this study was to examine the effects of a consumption of fluoride on osteoclast numbers appearing on the pressure side of alveolar bones at experimental tooth movement.

40 male rats were exposed to 0, 10, 25 mg/kg/day of sodium fluoride(NaF) in their drinking water for up to 60 days. Orthodontic appliance were activated to mesially tip maxillary first molar with 50- 70g. The rats were sacrificed at 1, 2, 4 days after initial activation. The number of osteoclast was counted in a 450 × 700 μm^2 area interradicular septum on the pressure side of the maxillary first molar.

The results were as follows,

1. There was significantly different osteoclast number between control group and 25 mg/kg/day group at all measured time. ($p< 0.05$)
2. There was significantly different active bone-resorption area between control group and 25 mg/kg/day group except at 96 hours post activation. ($p< 0.05$)
3. There was slight reduction of active bone- resorption area in control group from 48 hours to 96 hours but in both 10 mg/kg/day group and 25 mg/kg/day group a slight increase was observed from 48 hours to 96 hours.

Key words : orthodontic movement, fluoride, osteoclast

Orthodontic tooth movement requires remodelling of periodontal tissues, especially alveolar bone.[1-3] The first step in alveolar bone remodelling is resorption by osteoclasts.[4] Osteoclasts are essential to orthodontic tooth movement, requiring 1 to 3 days to appear after an initial appliance activation in the rat.[5-7]

Therefore intake of a substances or drugs inhibiting osteoclast-mediated bone resorption would result in delay of orthodontic tooth movement.[8]

The consumption of fluoride in drinking water has been shown to cause osseous as well as dental changes.[9] It has been found that, with incorporation of fluoride, bone becomes harder and less reactive, possibly because of a hardening and an increase in size of

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Fig 1. Orthodontic appliance and force application

the apatite crystal.[10,11]

Mitagi et al. demonstrated that the fluoride content of the rat alveolar bone was increased four times above that of the control group by addition of 100 ppm sodium fluoride to the drinking water for 90 days.[12]

Singer et al. reported the aspect of rat periodontal tissue during orthodontic tooth movement with 100 ppm fluoride intake for 90 days. They found that alveolar bone loss was considerably less in the fluoride group than in the non-fluoride group and undermining resorption was dominant in the fluoride group. Also, the degree of osteoclastic activity was far less in the fluoride group as compared with the non-fluoride group.[13]

It is unclear whether fluoride stimulate plasma cyclic AMP, which is well known as primary mediator encouraging cellular change accompanied by orthodontic force application.[14-16]

MATERIALS AND METHODS

1. Animals and orthodontic appliance

40 male Sprague-Dawley rats(28-35 days old) were purchased and acclimatized for 1 week to laboratory conditions. They were divided into three group of 12, 14, 14 animals respectively. The animals were housed, three or four per metal cage, at a constant temperature of 24℃ with commercial rat chow available ad libitum.

Animals in the experimental group received drinking water containing 10 and 25 mg/kg/day of sodium fluoride(NaF) each, whereas the control animals had tap water.

All experimental animals were given to drink daily for 60 days.

A lingual button was bonded to each rat occlusal surfaces of acid-etched maxillary first molar unilaterally with autopolymerizing orthodontic adhesives. Lower first and second molars were extracted. And then elastic powerchain connected between a lingual button on molar and an incisor. The powerchain was activated about 2mm, which delivered a force approximately 50-70g. (Fig. 1) The animals were sacrificed at 1, 2 and 4 days by excess ether inhalation.

2. Histologic preparation

The upper maxillary bones were excised immediately after sacrifice, and fixed in 10 % neutral formalin, decalcified in Plank-Rychlo's solution for three days, and embedded in Quetol-523M.

They were cut into mesiodistal series sections of 5 μm in thickness. The sections were stained with hematoxylin and eosin. Histological examination focused on the interradicular septum of buccal roots of the first molars. The number of osteoclasts was counted in a 450 × 700 μm^2 area of interradicular septum on the pressure side of the maxillary first molar. (Fig. 2)

Osteoclasts were identified as multi-nucleated cells, stained with eosin, containing round nuclei, and located immediately adjacent to the bone surface.[17] Individuals examining the slides were not informed of to which group they belong.

The degree of bone resoprtion was also measured according to the method described as below. (Fig. 3)

In the focused unit area, an interface between bony part and periodontal ligamental part was simply drawn as linear scale. And each ends of bone resorptive lac-

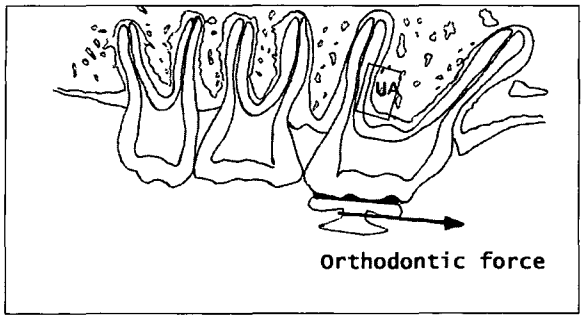

Fig. 2. A diagram showing experimental tooth movement and the focused unit area examined histologically. UA : Focused unit area

unae were dropped on the interface line. The percentage of total dropped line sum in the interface line was calculated and considered to represent the active bone-resorption area.

3. Data handling and statistical analysis

Mean and standard deviation were calculated for each variable for each time period.

Non-parametic test was performed to test for differences between groups, and a p value of less than 0.05 was considered statistically significant.

RESULT

1. The number of osteoclasts

Osteoclasts were observed at the interradicular septum on pressure side of the first molars in all three groups 24 hours after orthodontic activation.

Table 1 and Fig 4 shows the effects of fluoride comsumption on osteoclast numbers for 96 hours induced by experimental tooth movement.

Increase in the number of osteoclasts was observed in all three group with regard to the time of activation. 48 hours after activation, a two-fold increase in the osteoclast numbers observed comparing to 24 hours activation. But the number of osteoclasts after 96 hours was not as many as those of 48 hours activation in all three group.

A consumption of fluoride water containing 0 to 25 mg/kg/day of sodium fluoride(NaF) caused a dose-dependent decrease in osteoclast numbers.

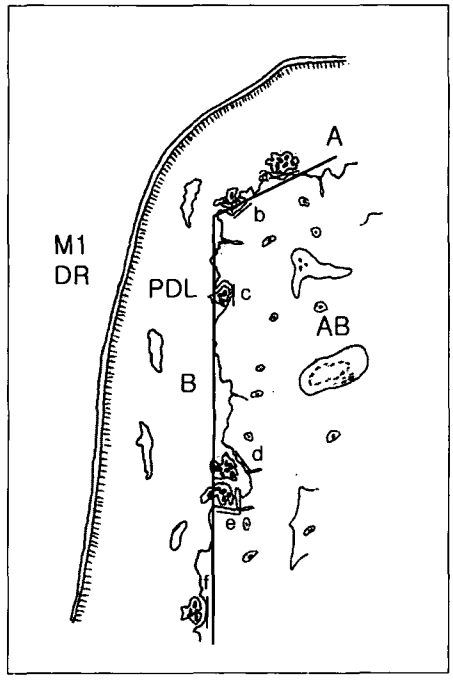

Fig. 3. The proposition of active bone-resorption area(%) = a + b + c + d + e + f / A + B
M1DR : Maxillary first molar distal root
PDL : Periodontal ligament
AB : Alveolar bone

There was significantly different osteoclast number between control group and 25 mg/kg/day group but not both between control group and 10 mg/kg/day group, and between 10 mg/kg/day group and 25 mg/kg/day group. ($p< 0.05$)

2. The proposition of active bone-resorption area

The proposition of active bone-resorption area is shown in Table 2 and Fig. 5.

A similar change is observed in the percentage of active bone-resorption area about time and group related changes as in an osteoclast cell number.

But, unlike in case of the cell number, the difference of active resorption area among the three groups, as a time being, reduced so that there was not significantly different even between control group and 25 mg/kg/day group at 96 hours activation. ($P < 0.05$)

There was slight reduction of resorptive area in

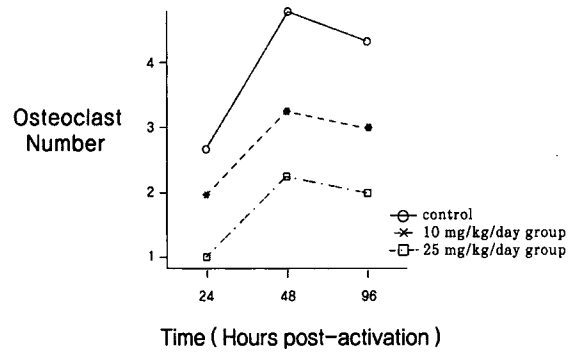

Fig 4. The number of osteoclasts in a control and experimental group

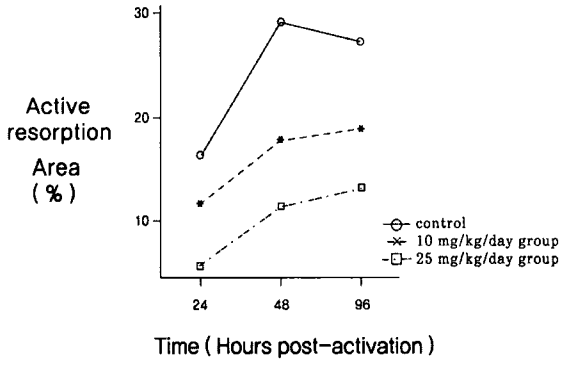

Fig 5. Active bone-resorption area in a control and experimental group

Table 1. The number of osteoclasts in a control and experimental group.

	Control group	*10 mg/kg/day group*	*25 mg/kg/day group*	*Kruskal Wallis test*	*Mann-Whitney test*		
	A	*B*	*C*		*A vs B*	*B vs C*	*A vs C*
24 Hrs.	2.67 ± 0.58	2.00 ± 0.82	1.00 ± 0.82	*	-	-	*
48 Hrs.	4.75 ± 1.50	3.25 ± 0.96	2.25 ± 0.96	-	-	-	*
96 Hrs.	4.33 ± 0.58	3.00 ± 1.00	2.00 ± 1.00	-	-	-	*

* : Significantly different at $P < 0.05$

Table 2. Active bone-resorption area in a control and experimental group(%).

	Control group	*10 mg/kg/day group*	*25 mg/kg/day group*	*Kruskal Wallis test*	*Mann-Whitney test*		
	A	*B*	*C*		*A vs B*	*B vs C*	*A vs C*
24 Hrs.	16.30 ± 3.63	11.78 ± 2.63	5.35 ± 6.23	*	-	-	*
48 Hrs.	29.15 ± 8.86	17.78 ± 4.50	11.30 ± 3.58	-	-	-	*
96 Hrs.	27.33 ± 3.06	18.80 ± 3.40	11.43 ± 8.02	-	-	-	-

* : Significantly different at $P < 0.05$

control group from 48 hours to 96 hours but in both 10 mg/kg/day group and 25 mg/kg/day group a slight increase was observed from 48 hours to 96 hours.

DISCUSSION

It is not clear whether the fluoride-treated animals may involve effects of fluoride on the number of osteoclasts. It is also not clear to what extent indirect effects of fluoride on osteoclastic activity may be responsible for the effects observed in vivo.[18]

Moreover, there is hardly any report about toxic or inhibitory effects of fluoride on the osteoclastic activity in alveolar bone.

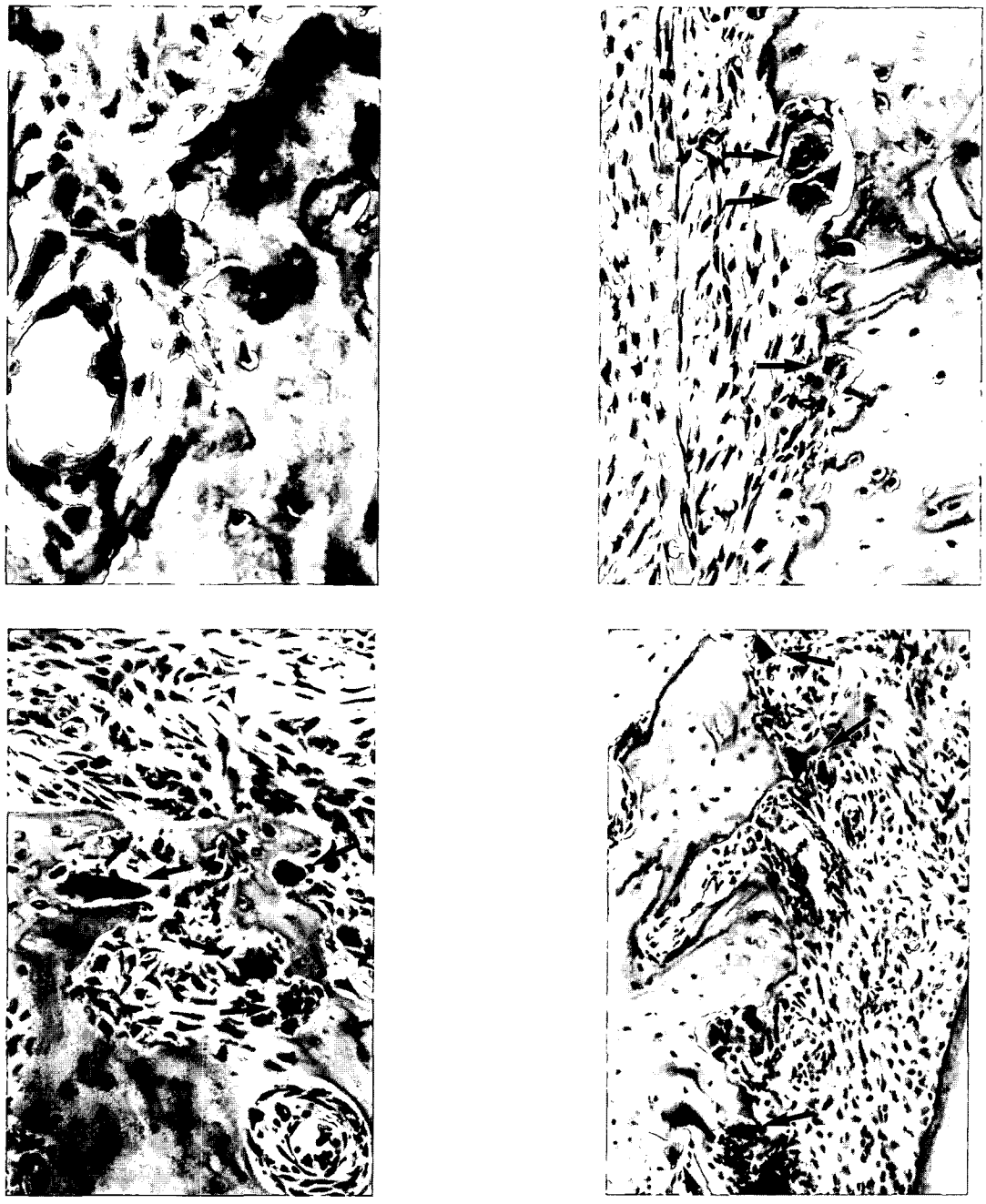

Fig 6. Histological changes in periodontal tissues. Arrows, osteoclast

(a) 24 hours after force activation of 10 mg/kg/day group (×400) Note an appearance of osteoclast right near the vessel on the alveolar bone surface

(b) 48 hours after force activation of control group (×100) Note a few numbers of multi-nuclaeated osteoclasts. Frontal resorption of alveolar bone is also observed.

(c) 96 hours after force activation of control group (×100) Note the extensive destruction of septal bone to lose bony entity.

(d) 96 hours after force activation of 25 mg/kg/day group (×40) Note a fusion of indirect bone resorption area and direct frontal bone resorption area on interseptal bone.

This study indicates that fluoride affects the number of osteoclast and active bone-resorption area at early stage in experimental tooth movement in rat molars.

Serum concentrations of fluoride were not measured in this study. However, our chosen dosages have proved to be effective in other studies.[10,12]

Osteoclasts were observed at 24 hours post activation in all three group though there was a small number of them along the limiting alveolar bone surface.

As an early response to orthodontically applied force, osteoclasts predominantly appeared in the alveolar bone within a day[20,21], even earlier by 6 hours.[22]

The number of osteoclasts was increased in all three group with regard to the time of activation until 48 hours. But 96 hours after activation, the number of osteoclasts was not as many as those of 48 hours activation in all three group.

There is a significantly different osteoclast number between control group and 25 mg/kg/day group at every time measured. ($p< 0.05$)

This reduced number of osteoclast in 25 mg/kg/day fluoride group is in accordance with the findings by Okuda and Kanehisa.[18]

They used 0.5mM and 1.0mM NaF as an experimental fluoride concentration and their results show no effect on osteoclast number at 0.5mM NaF. But, at 1.0mM NaF the number of osteoclast tend to decrease.

One explanation for the decrease in number could be that these concentration of fluoride are toxic for osteoclasts. With regard to NaF toxicity, studies on the effects of NaF on osteoblast proliferation and bone formation have shown that 1.0mM NaF decreased the proliferation of osteoblast-like cells and decreased colony formation[19].

An alternative explanation for the decreased number of osteoclasts could be an effect of fluoride on the attachment characteristics of the osteoclasts. This is currently under investigation by looking at adhesion structures of osteoclasts cultured in the presence of fluoride.

There is not significantly different osteoclast number between control group and 10 mg/kg/day group neither between 10 mg/kg/day group and 25 mg/kg/day group. ($p<0.05$) This indicates that the concentration of fluoride required to significantly affect appearance of osteoclast in the rat is at least 25 mg/kg/day. This concentration approximately comes up to 200 ppm of fluoridated water.

In fact, the dosages of fluoride in this work is considerably high compared with that ingested by children drinking fluoridated water. Such a high level had to be employed experimentally so that the distinctive effect was detectable inspite of short consumption period.

Therefore, some caution should be exercised in interpreting the results of this study in relation to higher concerntration of fluoride intake.

In this study, the percent of active bone-resorption area was briefly calculated by simply dividing total length of bone resorptive lacunae sum by focused alveolar bone-periodontal ligament interface.

This simple analytic method may not represent an exact quantitative resorption rate of alveolar bone in pressure side. However the change to increased resorptive lacunae between hours 24 and 96 could be confirmed by this formula.

Similar tendency as that in an osteoclast cell number is observed in the percentage of active bone-resorption area. One distinct difference would be that, as a time being, the difference of active resorption area among three group was slightly reduced.

There was not significantly different active bone-resorption area between control group and 25 mg/kg/day group at 96 hours activation. ($P < 0.05$)

This may be due to slight reduction of resorption area in control group from 48 hours to 96 hours but in both 10 mg/kg/day group and 25 mg/kg/day group slight increase was observed from 48 hours to 96 hours. (Fig 5)

These observation may reflect increased osteoclastic bone resorption in fluoride-treated animals in spite of reduced number of osteoclast.[23,24]

However, osteoclastic activity was decreased in in vivo studies of Faccini et al[25].

Recently Miyauchi et al. observed that osteoclast activity is inhibited by elevated extracellular levels of calcium. In this studies, the increase in extracellular calcium was augmented by fluoride.[26]

Because the clear correlation between orthodontically induced osteoclastic activity and the effects of fluoride is not yet demonstrated, it seems likely that more investigation on mechanism of fluoride effects upon periodontal tissue including alveolar bone should be carried out.

In this study, elastic powerchain(or elastomeric chain) was used as a source of orthodontic force.[27] In other previous studies, elastic module[1] or closed type coil spring[5,28] were dominantly used as an orthodontic force in the rat.

Elastomeric chains experience a rapid loss of force due to stress relaxation, especially an initial loss of 40-70 % force occured within 24 hours.[29-31] In this study, however, observation period was up to 96 hours, an initial phase of tooth movement.[32] Therefore, force decayed characteristic of elastomeric chain would not influence on aspect of tooth movement in this study.

CONCLUSION

40 male rats were exposed to 0, 10, 25 mg/kg/day of sodium fluoride(NaF) in their drinking water for up to 60 days. Orthodontic appliance were activated to mesially tip maxillary first molar with 50-70g. The rats were sacrificed at 1, 2, 4 days after initial activation. The number of osteoclast was counted in a 450 × 700 μm^2 area interradicular septum on the pressure side of the maxillary first molar.

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국문초록

백서에서 불소의 투여가 실험적 치아이동시 파골세포에 의한 골흡수에 미치는 영향

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김 도 훈* · 문 윤 식* · 고 재 승** · 김 현 만**

교정적인 치아이동은 치주조직, 특히 치조골의 개조를 필요로 한다. 불소는 파골세포에 의한 골흡수를 방해하는 물질로 알려져 있다.

이 연구의 목적은 불소의 섭취가 교정적인 치아이동시 압박측 치주조직에서 파골세포의 출현에 어떠한 영향을 끼치는 지를 조사하는 것이다.

40마리의 백서에게 60일동안 불화나트륨(NaF)가 각각 0, 10, 25 mg/kg/일 함유된 물을 음수시킨 후, 약 50- 70g의 교정력을 가하여 상악 제 1 대구치를 근심쪽으로 경사이동시켰다.

교정력을 가한 24, 48, 그리고 96 시간 후에 대조군과 실험군을 희생시킨 후 조직편을 제작하여 상악 제 1 대구치의 압박측 치주조직 450 × 700 μm^2을 관찰하여 출현한 파골세포의 수와 흡수량을 조사하여 다음과 같은 결론을 내렸다.

1. 모든 측정시간에서 25 mg/kg/일 군에서 대조군보다 파골세포의 수가 유의성 있게 적었다.($p< 0.05$)
2. 24, 48시간 측정에서 25 mg/kg/일 군에서 대조군보다 흡수되는 면적이 유의성 있게 적었다.($p< 0.05$)
3. 대조군에서는 48시간에서 96시간 사이에 흡수되는 면적이 약간 감소하였으나, 10 mg/kg/일 군과 25 mg/kg/일 군 모두에서는 약간 증가하였다.

주요 단어 : 교정적 치아이동, 불소, 파골세포