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Periodontal 5′-deiodination on forced-induced root resorption—the protective effect of thyroid hormone administration

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SUMMARY The present investigation was designed to study the protective effect given by thyroid hormone (TH) on root resorption: (1) whether intra-peritoneal versus oral TH administration had the same efficiency; and (2) whether this effect involved local or systemic mechanisms. For this purpose, circulating T3 levels, systemic alkaline phosphatase (APase) activity, and 5′deiodinase (5′D) activity were evaluated in the periodontal area of 80 Sprague–Dawley rats, 8 weeks of age, in which orthodontic appliances had been inserted.

The results showed that TH-treated animals (intra-peritoneal or oral) had significantly less force-induced root resorptive lesions compared with a control group, without apparent changes in T3 or APase levels, and that periodontal remodelling was accompanied by a significant increase in local T3 generation as a result of T4 deiodination. This 5′D activity was higher in those animals that received exogenous TH. These results suggest that this protective TH mechanism may be achieved at a local level and that administration of low doses of TH may play a protective role on the root surface either during orthodontic treatment or in those patients that present spontaneous root resorptive lesions.

Introduction

Thyroid hormone (TH) plays a crucial role in normal growth and development of vertebrates. It is generally accepted that these effects are mainly mediated by the interaction of T3 with its nuclear receptor, whereas T4 acts as a prohormone for local T3 neogenesis catalysed by 5′-deiodination (5′D) reactions. Other enzyme reactions that perform T4 5′D represent an inactivation pathway, since T4 is converted into reverse T3 (rT3), a hormone without biological activity (Larsen *et al*., 1981). These enzymes, called deiodinases, show different developmental profiles, species, and tissue-specific expression, and a regulation dependent on physiological and pathophysiological factors. For these characteristics, the deiodinases have been considered as gatekeepers of TH action at organ-specific level (Kohrle, 1996).

Bone is a dynamic tissue that constantly undergoes remodelling. Remodelling is a coupled process in which bone resorption is normally followed by new bone formation as a result of the interaction of nutritional, genetic, and hormonal factors (Roodman, 1996). TH is involved in bone development and maturation. It is critical for cartilage growth and differentiation, enhances the response to growth hormone, and stimulates bone resorption. TH acts directly on bone remodelling by stimulating osteoblast–osteoclast coupling, but it also has an indirect effect via some growth factors that are closely related to bone metabolism, such as insulin-like growth factor I (IGF-I), which is locally produced in several bone cells by TH action (Wakita *et al*., 1998). In the specific case of teeth, TH action has been recorded in bone resorption occurring in the periodontal region and in the apical roots (Loberg and Engström,

1994). Previous reports have shown that administration of low thyroxine doses decreases the extent of force-induced root resorption in both humans (Loberg and Engström, 1994) and rats (Poumpros *et al*., 1994), suggesting that thyroid function is an important clinical factor in the aetiology of force-induced root resorption. The present study was designed to further clarify whether in the TH-root resorption protective effect: (1) intra-peritoneal versus oral TH administration had the same efficiency; and (2) this protective mechanism involved local or systemic mechanisms.

Material and methods

Reagents

Non-radioactive thyronines were obtained from Sigma Chemical Co., St Louis, MO. Radiolabelled thyronines (^{125}I-T3 and ^{125}I-rT3; SA, 1250 and 1174 μCi/μg, respectively) were purchased from New England Nuclear, Boston, MA. Paracetamol was obtained from Bayer-México, México, DF; and the phosphatase alkaline kit from Merck-México, México, DF. All other reagents were of the highest purity commercially available.

Animals

The study was conducted on 80 male Sprague–Dawley rats (250 ± 18 g bw; 8 weeks of age). The animals were housed in an automatically controlled environment (21 ± 1°C; 12-hour light–dark cycle). They were fed a standard pellet diet (PMI, Brentwood, MO) and tap water *ad libitum*. All animals were handled according to the International Regulations of Laboratory Animal Care. All animals received a daily 20-mg dose of paracetamol in tap water to avoid possible pain secondary to orthodontic appliance insertion. All the rats were weighed at the beginning of the experiment and before they were killed, and all showed a similar increase in body weight.

Experimental protocol

The animals were divided into the following experimental groups ($n = 16$): C, control intact animals daily-injected, with an intra-peritoneal (i.p.) saline solution; C + TH, control animals with oral TH administration; AP, animals with orthodontic apparatus; AP + iTH, animals with apparatus plus i.p. TH administration; AP + oTH, animals with orthodontic apparatus plus oral TH administration.

Orthodontic apparatus

The appliance consisted of a wire loop anchored to the maxillary incisors with a wire ligature passing through a hole drilled in these teeth and cemented with glass-ionomer cement under ether anaesthesia (see Figure 1). The appliance was designed to exert a 50-g lateral force over the maxillary incisors. This force value was chosen from previous studies where it has been reported that root resorption occurs within 7 days after the application of force magnitude (Poumpros *et al*., 1994; Vandevska-Radunovic

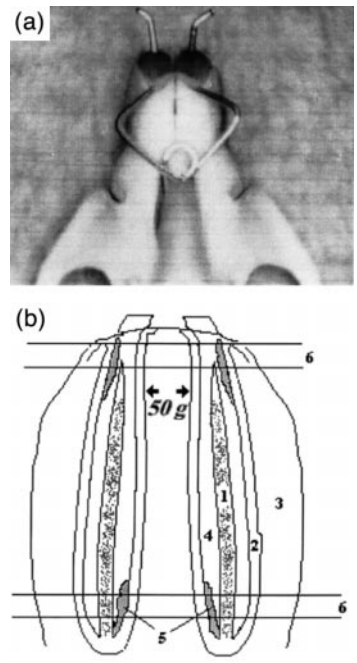

Figure 1 (a) Photograph of appliances (wire loop) *in situ*. (b) Diagram of root sections. 1, Dental pulp; 2, periodontal ligament; 3, bone; 4, dentine; 5, resorption zones; 6, areas used for evaluation (1 mm). Arrows indicate the force direction.

et al., 1997). The force was adjusted with a Dendrix gauge before the loop was placed.

TH treatment

Intra-peritoneal daily treatment contained 4 μg/kg of T4 and 400 ng/kg of T3 in a 200 μl saline solution. This dose was chosen taking into account previous studies where this amount was effective in reducing surface resorption (Poumpros *et al*., 1994). Oral daily treatment contained 8 μg/kg of T4 and 800 ng/kg of T3 in 200 μl of 10 per cent sugar solution. This dose was chosen as these hormones are 40 per cent absorbed by the oral epithelium within the first 10 minutes of application (Haynes and Murad, 1980). Sugar solution was used as a vehicle so that the rats accepted the oral administration of 200 μl of TH solution and all the volume could be swallowed. TH administration began 2 days before the orthodontic appliances were inserted. Force activation was undertaken daily between 10 and 11 am.

Experimental procedure

Tooth resorption results from injuries to or irritation of the periodontal ligament (PDL) and/or tooth pulp, and APase activity is considered to show the state of bone metabolism at the systemic level. In the specific case of TH, 5'D activity represents a local source of T3, whereas T3 circulating levels reflect the systemic thyroid status. All animals were killed by decapitation after 10 days of hormone treatment. Serum was analysed for T3 levels and APase activity. In eight animals from each group, maxillary incisor peri-alveolar tissue was dissected and immediately frozen in acetone–dry ice for deiodinative determination. In the remaining eight animals, the premaxilla/maxilla were dissected after decapitation and kept for 2 days in 10 per cent formol, followed by 2 days in 5 per cent nitric acid for decalcification.

Although root resorption was observed in different areas along the length of the root, the most evident lesions occurred in the root zone contralateral to the force application (pressure zones, see Figure 1). A 1-mm sample of each zone (apical and gingival) was mounted in paraffin and serially sectioned, 40 μm thick, in a transverse plane (frontal). Three sections from each zone were collected and stained with haematoxylin/eosin (Bancroft and Cook, 1979). Sections were analysed using a light microscope (Leica, Heerbrugg, Switzerland). The root surface perimeter, as well as the extent of root surface resorptive lesions, was determined using a 5×5 mm square grid projected in the viewing field at ×32 magnification. The root perimeter was measured by counting the number of grid squares covering the root outline. The amount of root resorption was determined by counting the number of squares with root surface lesions and comparing that number with the number of squares of the total root surface. Three consecutive sections and two different areas of each animal were analysed.

Circulating T3 and APase activity

Serum T3 levels were measured by homologous radio-immuno assay (RIA) methods. This RIA was adapted to the rat by adding homologous hypothyroid serum to the standard curve (Valverde and Aceves, 1989). The lower sensitivity limit was 12.5 ng/dl, and the intra- and inter-assay coefficients of variation were 9.0 and 12.8 per cent, respectively. Serum APase activity was assayed with an APase kit (Merck-México).

Enzymatic assay

Total 5'D was determined by a modification of the radiolabelled iodide release method (Valverde and Aceves, 1989). Briefly, tissues were homogenized in HEPES buffer (10 mM HEPES, 0.32 M sucrose, 1.0 mM EDTA and 10 mM DTT, pH 7.4) and centrifuged at 2800 g, for 30 minutes at 4°C. Assay conditions were as follows: 200 μg of protein, 2 nM ^{125}I-rT3, and 20 mM DTT. Released acid-soluble radio-iodide was isolated by chromatography on Dowex 50W-X2 columns after a 3 hour incubation at 37°C. Proteins were measured by the Bradford method (Bio-Rad protein assay, Bio-Rad, Richmond, CA). The results were expressed as femtomoles of radio-iodide released per milligram of protein/hour.

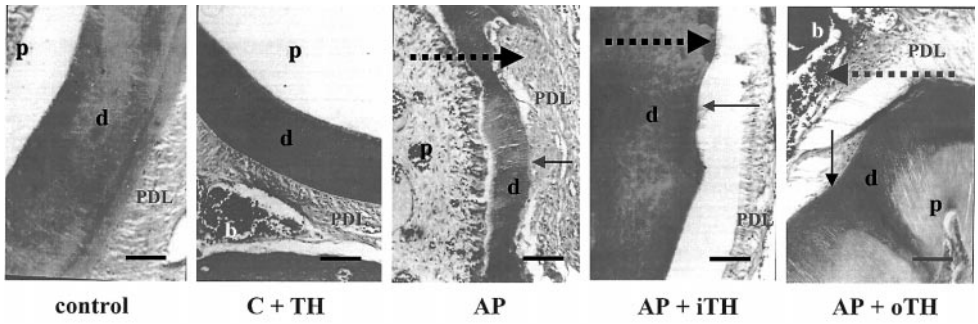

Figure 2 Histological illustration showing orthodontic root resorption (thin arrow) in control and TH treated animals. Control, intact animals plus i.p. saline solution; C + TH, control plus oral TH treatment; AP, animals with orthodontic appliances; AP + iTH, orthodontic appliances plus i.p. TH treatment; AP + oTH, orthodontic appliances plus oral TH treatment. p, dental pulp; d, dentine; b, bone; PDL, periodontal ligament, dashed arrow indicates the direction of tooth movement. Bar = 0.1 mm.

Statistical analysis

Data were expressed as mean ± SD. Differences between the experimental groups were analysed using one factor ANOVA and Tukey's HSD test. Differences greater than $P < 0.05$ were considered statistically significant.

Results

Lateral movement of the maxillary incisors was apparent in all groups where a force was applied. The amount of expansion in these groups was similar (2.8–3.4 mm). Figure 1 shows a photograph of the appliances *in situ* and the root areas that were analysed. Three sections of each tooth were analysed and two regions of each section were used to determine the extent of root surface resorptive lesions. Figure 2 shows a representative macroscopic appearance of an undecalcified section of maxillary bone and resorption root area of each group. No root surface lesions were found in C and C + TH animals. In the AP group, marked lesions were observed (28 per cent); whereas in the TH-treated groups (AP + iTH and AP + oTH), the root surface lesions were significantly less (16 and 18 per cent, respectively) compared with the control groups. The values of root surface lesions are summarized in Figure 3. Circulating levels of T3 (Figure 4) and APase values (Figure 5) were similar in all groups. Figure 6 shows the

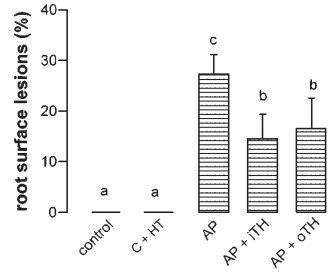

Figure 3 Root surface lesions (percentage) in control and TH treated animals (see details in Materials and methods). Control, intact animals plus i.p. saline solution; C + HT, control plus oral TH treatment; AP, animals with orthodontic appliances; AP + iTH, orthodontic appliances plus intra-peritoneal TH treatment; AP + oTH, orthodontic appliances plus oral TH treatment. Values represent the mean ± SD ($n = 8$). Means with different letters are significantly different ($P < 0.05$).

values of 5′D activity obtained in the periodontal region. 5′D activity in the AP group was significantly higher in comparison with the C group. However, rats with appliances plus TH treatment (AP + iTH and AP + oTH) showed the highest 5′D activity.

Discussion

The present study shows that application of a lateral force of 50 g over the maxillary incisors was accompanied by lateral tooth movement and root resorption lesions, as well as a significant

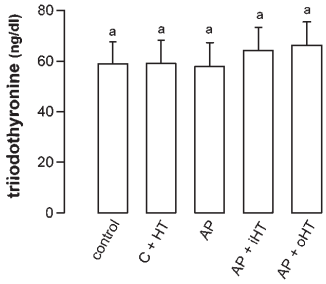

Figure 4 Circulating T3 levels in control and TH-treated animals. Control, intact animals plus i.p. saline solution; C + HT, control plus oral TH treatment; AP, animals with orthodontic appliances; AP + iTH, orthodontic appliances plus intra-peritoneal TH treatment; AP + oTH, orthodontic appliances plus oral TH treatment. Values represent the mean ± SD ($n = 16$). Means with different letters are significantly different ($P < 0.05$).

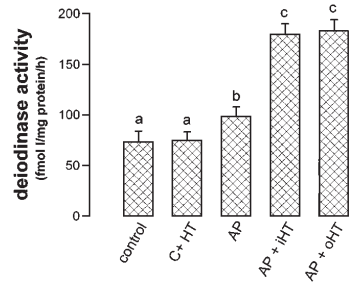

Figure 6 Periodontal ligament 5'D activity in control and TH-treated animals. Control, intact animals plus i.p. saline solution; C + HT, control plus oral TH treatment; AP, animals with orthodontic appliances; AP + iTH, orthodontic appliances plus intra-peritoneal TH treatment; AP + oTH, orthodontic appliances plus oral TH treatment. Values represent the mean ± SD ($n = 8$). Means with different letters are significantly different ($P < 0.05$).

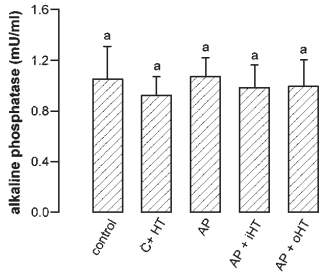

Figure 5 Serum alkaline phosphatase levels in control and TH-treated animals. Control, intact animals plus i.p. saline solution; C + HT, control plus oral TH treatment; AP, animals with orthodontic appliances; AP + iTH, orthodontic appliances plus intra-peritoneal TH treatment; AP + oTH, orthodontic appliances plus oral TH treatment. Values represent the mean ± SD ($n = 16$). Means with different letters are significantly different ($P < 0.05$).

increase in 5'D activity in the periodontal region without apparent changes in circulating T3 and APase levels, suggesting that orthodontic movement has consequences only at a local level. These data are in contrast with those found by Poumpros *et al.* (1994) in a similar model, where rats with appliances showed root resorption lesions and significantly lower levels of T4 and APase. They concluded that tooth expansion systemically modified bone metabolism and that the decrease in T4 could be the result of stress induced by this manipulation. One plausible explanation, at least with regard to TH, could be the occurrence of fasting periods secondary to pain and discomfort induced by the appliances. Both studies used the same force (50 g) at similar intervals (10 days), but the rats in the present study received paracetamol in the tap water and body weight gain was similar in all groups. A similar analysis was not mentioned in the work of Poumpros *et al.* (1994) but it is well known that fasting decreases circulating levels of TH (Larsen *et al.*, 1981). The results in the present investigation are the first report showing increases in periodontal 5'D associated with appliances, which is a clear indication that tooth remodelling is accompanied by local T3 generation. Although the mechanism by which deiodination is regulated at periodontal level was not evaluated, the clear-cut influence of the sympathetic nervous system over deiodinative regulation in other tissues is suggestive (Aceves *et al.*, 1999; Kohrle, 1999). Experimental evidence clearly indicates that tooth innervation is implicated in the modulation of remodelling reactions by playing not only a sensory role, but also as part of local effector mechanisms. Effectively, after appliances are inserted, a pronounced sprouting response of nerve fibres from sensory and sympathetic origins is observed in periodontal and pulp tissue, modulating a

variety of tissue reactions, such as increased blood flow, cellular extra-vasation, bone resorption, and alveolar bone remodelling (Vandevska-Radunovic *et al*., 1997).

The second finding in this study was that independent of the administration route, TH-treated animals showed significantly less root resorption lesions and higher periodontal 5′D activity without apparent changes in systemic variables. These data corroborate the finding that administration of lower doses of TH reduces force-induced root resorption lesions (Loberg and Engström, 1994; Poumpros *et al*., 1994; Shirazi *et al*., 1999) and suggest again that these effects are achieved at a local level. The significant increase in periodontal 5′D activity without apparent changes in circulating T3 levels may indicate that tissues under injury or irritation are capable of sensing slight changes in the TH available. Although controversial, similar effects have been described in other circumstances, as in the case of children with attention deficit hyperactivity disorder, in which treatment with supra-physiological (but not sufficient to produce hyperthyroidism) doses of TH had a beneficial effect on their behaviour, specifically on hyperactivity and impulsivity (Hauser *et al*., 1993; Weiss *et al*., 1997).

The specific mechanisms by which TH may modulate tooth remodelling have not been studied; however, the finding that the amount of expansion in all groups was similar is suggestive that a TH mechanism may be involved in the protection of cementum and dentine of osteoclastic resorption. Although further studies to corroborate this hypothesis will be undertaken, some indirect evidence exists. It is well documented that TH exerts a bi-phasic effect on bone formation and resorption. At lower doses, TH may increase bone formation; whereas at high levels, bone resorption is enhanced (Baran, 1996). Moreover, data on tooth remodelling have shown that hypothyroidism is accompanied by a reduction in the resistance of tooth roots to resorb (Sismanidou *et al*., 1996), whereas in hipo-physectomized rats, the administration of thyroxine results in a significant increase in dentine apposition and longitudinal tooth growth (Hansson *et al*., 1978).

Conclusions

The present results suggest that a protective TH mechanism may be achieved at local level and that administration of low doses of TH might provide a protective role on the root surface during orthodontic movement, and in those patients that present spontaneous root resorption lesions.

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