Bisphosphonate-induced delayed tooth eruption in Osteogenesis Imperfecta children.

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ABSTRACT

Bisphosphonates are currently used in the therapy of osteogenesis imperfecta (OI) to decrease the bone fragility observed in OI patients. Bisphosphonate therapy delays tooth eruption in rats. The aim of this study, therefore, was to determine whether or not bisphosphonate therapy similarly delays tooth eruption in children. The clinical emergence of teeth was observed by children's parents or by physicians. The calculated dental age and the number of delayed teeth were determined for 33 bisphosphonate-treated and eight untreated OI patients, and for strictly sex- and age-matched controls. There were significant differences between bisphosphonate-treated patients and controls for calculated dental age ($p<0.001$) and number of delayed teeth ($p<0.001$). No significant differences were observed between untreated OI patients and control subjects. Bisphosphonate therapy was associated with a mean delay of 1.67 year in tooth eruption in children with OI.

INTRODUCTION

Osteogenesis imperfecta (OI) is an inherited disorder of the connective tissue caused by mutations in either the collagen Iα1 gene on chromosome 17 or the collagen Iα2 gene on chromosome 7 (Sykes *et al*., 1986). The reported incidence is between 6 and 20 per 100,000 newborns (Andersen and Hauge, 1989). The disease is usually inherited in an autosomal dominant manner, although *de novo* mutations are common. Patients with OI may show any of a range of signs related to defective collagen, including bone fragility, growth deficiency, blue sclerae, hearing loss, joint laxity, and dentinogenesis imperfecta type I and other dental abnormalities such as agenesis (Engelbert *et al*., 1998), apically extended pulp chambers (Lunkinmaa *et al*., 1987), impaction (Schwartz and Tsipouras, 1984; O'Connell and Marini, 1999), invagination (Lukinmaa *et al*., 1987) and denticles (Lunkinmaa *et al*., 1987; Lund *et al*., 1998; Lindau *et al*., 1999). Tooth eruption was age-appropriate in all but one of the 68 index patients recently studied by Malgrem and Norgren (Malmgren and Norgren, 2002) and in all 27 OI patients described in a previous study (O'Connell and Marini, 1999).

Bisphosphonates are currently used to decrease bone fragility in OI patients. Bisphosphonates have the unique property of inhibiting the ability of osteoclasts to resorb

bone *in vitro* (Piper *et al*., 1994) and in long bones *in vivo* (Reitsma *et al*., 1980). Studies of osteopetrotic rodents suggest that localized alveolar bone resorption is a requirement before tooth eruption (Tiffee *et al*., 1999). Bisphosphonates inhibit bone resorption and thereby delay tooth eruption of both molars and incisors in rats (Grier and Wise, 1998).
We studied OI patients to determine whether or not tooth eruption was delayed by bisphosphonate therapy.

MATERIALS AND METHODS

The study population consisted of children with OI who were registered in patient groups (Association de l'Osteogenese Imparfaite, Association Francophone Belge de l'Osteogenese Imparfaite). A child was included if any one of the permanent teeth had fully emerged. The first group of patients was composed of 33 children, 6.2 to 14.6 years old, receiving nitrogen-containing bisphosphonate therapy (12 boys and 21 girls). Sex- and age-matched controls were recruited from the French general population. The second group of patients was four boys and four girls with OI (ages were 5.8 to 13.4 years) who had never received bisphosphonate, and age- and sex-matched bisphosphonate-treated OI patients. Sex- and age-matched controls used for the second group were the same as those used for the first group. The patients in the two groups were classified as OI types I, III and IV as defined by Sillence (Sillence *et al*., 1979).
This study protocol was approved by a local Ethics Committee, and informed consent was obtained from the mother or the father of the children.
Each child underwent a visual inspection under natural light for the presence of permanent and deciduous teeth. All inspections were performed by physicians or parents, not by dentists. The status of each tooth was recorded on a diagram of the permanent dentition using a scale of 0-1: 0, no tooth present; 0.5, crown of the emerging tooth less than half emerged; 1, tooth fully emerged and in occlusion.
The data published by Hurme (Hume, 1949) were used to determine the number of delayed teeth.
Two different methods were used to assess dental age. Method 1 was based on the number of permanent teeth that had emerged; data were collected for girls and boys between 5.8 and 12.9 years of age (Hagg and Taranger, 1985). Method 2 used a mathematical model that took several variables into account to calculate the age as a continuous variable (Foti *et al*., 2003); this model was designed to be used in the absence of radiographs, when only data from clinical intra-buccal examinations are available. The dental age was estimated using the following formula:
Estimated age =13.652

-(0.514 X number of erupted deciduous upper incisors)
-(0.236 X number of erupted deciduous upper molars)
+(0.314 X number of erupted permanent upper canines)
-(1.748 X number of erupted permanent upper first molars)
+(1.012 X number of erupted permanent upper second molars) +(0.994 X number of erupted permanent upper third molars)

+(0.252 X number of erupted lower premolars)
+(0.285 X number of erupted permanent lower second molars)
+(1.537 X number of erupted permanent lower third molars)

This method can be used only for children in who the first permanent molar has already erupted.
The paired t-test and chi-2 test were used for statistical analyses.

RESULTS

We assessed the dental ages of bisphosphonate-treated OI children and compared them to those of a healthy control group and to a group of OI patients who had not received bisphosphonate therapy.
The accuracy of assessment of chronological age by analysis of tooth emergence was checked in the control group. For Method 1, there was no significant difference between the calculated dental age and the chronological age (paired t-test t=0.60). However, a significant difference was observed between the dental age calculated by Method 2 and the chronological age (paired t-test t=5.00, p<0.001).
Figure 1 shows chronological age and dental age calculated by the method 1 (Hagg and Taranger 1985) for all the children. The mean (±SEM) tooth eruption delay was 0.009±0.07 for controls and 1.67±0.40 for bisphosphonate-treated OI patients (p«0.001). Table 1 reports the number of delayed teeth in OI patients and matched controls: there were significant differences between controls and both group 1 (p<0.01) and group 2 (p<0.01) as assessed by the chi-2 test.
Patients and controls in group 1 (Table 2) showed significant differences concerning (i) the dental age determined by Method 1 (paired t-test t=7.00, p«0.001), (ii) the dental age determined by Method 2 (paired t-test t=3.21, p<0.01), and (iii) the number of delayed teeth (paired t-test t=3.87, p«0.001) (Table 3).
In contrast, no difference was observed for the same items between untreated OI patients and control subjects in group 2 (Table 2).

DISCUSSION

For practical and ethical reasons, our study was based only on clinical examination of dental eruption status. This study does not address the numerous oral problems associated with OI, such as type III dental malocclusion, posterior crossbites or impacted teeth (Schwartz and Tsipouras, 1984). Although various dental abnormalities are associated with OI, alterations of tooth eruption were not found to be prevalent in a previous study (Malmgren and Norgren, 2002). Bisphosphonate therapy was not administered to the patients described in this previous study and only one of the 68 patients included had delayed tooth eruption; in this patient, only the lower medial incisor had erupted at 19 months of age, and at 30 months of age only eight teeth had erupted, although all expected teeth could be found on radiographs. Thirty-two patients in this study were between 5 and 14 years old and had age-appropriate tooth eruption. An earlier study involving about 40 patients reported similar findings (O'Connell and Marini, 1999).
We also observed that there is no difference for tooth eruption between untreated OI patients and sex- and age-matched controls (Table 2); our comparison of 8 bisphosphonate-treated OI patients with 8 controls, despite the small numbers, revealed significant differences for number of permanent teeth and dental age (determined by method 1 described above) (Table 2).
An overall picture of the dentition as assessed by analysis of tooth emergence can only be obtained by counting emerged teeth in individuals during the period of emergence of the primary or permanent dentition (Hagg and Hagg, 1986). Various methods have been reported by many investigators (Lysell *et al.*, 1962; Filipsson, 1975, Moorrees and Kent, 1978; Hagg and Taranger, 1985). We only used a clinical analysis of tooth emergence. Tooth eruption is mediated by bone remodeling and dental development which is contingent on further root development prior to initiation of bone remodeling. Our clinical examination does not distinguish between bone remodeling and root development. Nevertheless, there is biological evidence that bisphosphonates have a direct effect only on the former mechanism (Marks *et al.,* 1995).
The development of a tooth and its emergence into the oral cavity are complicated phenomena, and the mechanisms are not yet fully understood. Individual teeth develop within the jaws, and eruption occurs during a complex process involving bone remodeling on

opposite sides of the developing teeth (Marks *et al.,* 1995). In addition to bone changes, eruption of the permanent teeth necessitates resorption and exfoliation of the primary teeth. The requirement of alveolar bone resorption for tooth eruption was first noted in osteopetrotic rodents. Ostepetrosis is a congenital bone disease involving reduced bone resorption but not reduced bone formation (Marks, 1973), and in many cases there is failure of teeth eruption. For example, in the toothless rat (*tl*), the teeth are fully formed but do not erupt (Cotton and Gaines, 1974). Such animals have few osteoclasts, and they are probably non-functional (Marks, 1973; Seifert *et al.,* 1988). This was confirmed by scanning electron microscopy that showed the absence of bone resorption in the crypts of *tl* rats, contrasting with the scalloped crypt surfaces in normal rats, which indicate bone resorption. In another strain of osteopetrotic rats (*ia*) in which the teeth do not erupt, irradiation and injection of spleen cells from normal rats resulted in tooth eruption as a result of the formation of functional osteoclasts (Marks, 1981). Also, osteopetrotic (*op/op*) mutant animals have fewer osteoclasts than normal mice, and their teeth do not erupt (Marks and Lane, 1976).
A direct causal relationship between resorption of alveolar bone by osteoclasts and tooth eruption has been shown in studies in which pamidronate, a bisphosphonate that reduces bone resorption by osteoclasts, was injected into rats (Grier and Wise, 1998): eruption was delayed by 8 days for the first mandibular molars, 1.6 days for the mandibular incisors and 2.5 days for the maxillary incisors. The cytological effect of this treatment was to increase the size of the osteoclasts, and the number of nuclei, suggesting that the osteoclasts might be increasing in size to compensate for their reduced ability to resorb bone (Grier and Wise, 1998). It was also found (Alatli and Hammarstrom, 1997) that both single and multiple injections of a bisphosphonate in rats inhibited the formation of acellular cementum in the teeth; a cellular hard tissue matrix was formed on the newly developed dentin surface, but not on previously formed acellular cementum. This hard tissue matrix was gradually resorbed after ending bisphosphonate treatment (Wesselink and Beertsen, 1989; Wesselink and Beertsen, 1994).
Nitrogen-containing bisphosphonates (N-BPs) are currently used in the treatment of bone fragility in humans. The only previously reported effects of bisphosphonates on teeth are slight disruptions in the formation of enamel and dentine in rats (Fouda *et al.,* 1991). After internalization, N-BPs inhibit a key enzyme, farnesyldiphosphate synthase, in the biosynthetic mevalonate pathway (Green, 2004; Papapoulos, 2006). As a result, N-BPs interfere with a variety of cellular functions essential for the bone-resorbing activity and survival of osteoclasts (Russell *et al.,* 1999). Several intermediates in this pathway, including farnesyl pyrophosphate and geranylgeranyl pyrophosphate, are required for the posttranslational modification (i.e. prenylation) of guanosine triphosphate-binding proteins such as Ras, Rho and Rac (Luckmann *et al.,* 1998). These signaling molecules are involved in the regulation of cell proliferation, cell survival and cytoskeletal organization (Russell *et al.,* 1999). In particular, inhibition of protein prenylation and Ras signaling within osteoclasts leads to defects in intracellular vesicle transport (Alakangas *et al.,* 2002). As a result, osteoclasts cannot form a tight-sealing zone or ruffled borders, which are required for bone resorption. These observations may explain the delayed tooth eruption observed in bisphosphonate-treated OI patients.

Our study shows that bisphosphonate therapy delays tooth eruption in humans, consistent with its effects in rats. Bisphosphonates have a large effect on bone fragility in OI patients and delayed tooth eruption is a minor side-effect of this therapy. However, delayed tooth eruption should be considered, particularly as it may increase the number of impacted teeth in patients already suffering from dental disorders.

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TABLES

Table 1: Number of delayed teeth in group 1 and group 2. Criteria used were from Hurme (Hurme, 1949). Bisphosphonate-treated osteogenesis imperfecta patients (OI-BP); Osteogenesis Imperfecta patients (OI); controls (C)

Table 2: Tooth eruption in group 1 and group 2: statistical comparisons. Values of t in paired t-tests. p values are in square brackets. NS=not significant. ND=not determined

FIGURE

Figure 1: Overview of the individual results. Dental maturity was determined as the difference (plotted on the ordinate scale) between estimated dental age (method 1, see methods) and true chronological age(abcissa). Bisphosphonate-treated osteogenesis imperfecta patients (OI-BP); controls (C)

Number of delayed teeth	GROUP 1		GROUP 2		
	OI-BP	Controls	OI-BP	OI	Controls
0	17	31	2	6	7
1	5	1	1	1	0
2	4	1	2	0	1
≥3	7	0	3	1	0

Table 1: Number of delayed teeth in group 1 and group 2. Criteria used were from Hurme (Hurme, 1949) Bisphosphonate-treated osteogenesis imperfecta patients (OI-BP); Osteogenesis Imperfecta patients (OI); controls (C)

	GROUP 1	GROUP 2	
	Bisphosphonate-treated OI patients versus controls	Untreated OI patients versus controls	Bisphosphonate-treated OI patients versus controls
Number of pairs	33	8	8
Number of permanent teeth	7.32 [«0.001]	1.72 [NS]	3.35 [<0.01]
Number of delayed teeth	3.87 [«0.001]	0.75 [NS]	3.07 [<0.02]
Dental age method 1	7.00 [«0.001]	1.74 [NS]	3.96 [<0.01]
Dental age method 2	3.21 [<0.01]	ND	ND

Table 2: Tooth eruption in group 1 and group 2: statistical comparisons. Values of t in paired t-tests. p values are in square brackets. NS=not significant. ND=not determined

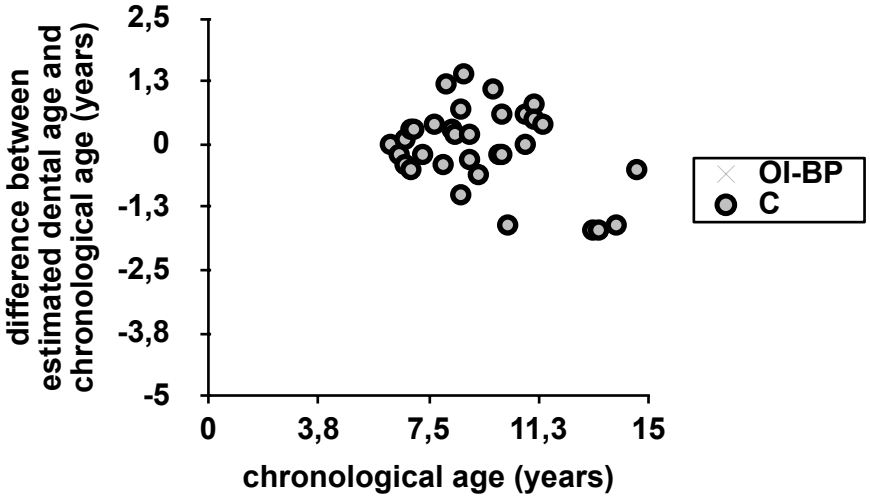

Figure 1: Dental maturity was determined as the difference (ordinate scale) between estimated dental age (method 1, see methods) and true chronological age (abcissa)