Original Article

Effects of Orthodontic Force on Osteoblasts and Alkaline Phosphatase

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ABSTRACT

Acid and alkaline phosphatase changes have been demonstrated histo-chemically and biochemically during the early stages of an orthodontic tooth movement cycle. However, quantitative data on neither alveolar bone nor serum phosphatase changes over an entire tooth movement cycle have been reported. This study examined acid, tartrate-resistant acid (TRAP), and alkaline phosphatase changes in serum and alveolar bone during an orthodontic tooth movement cycle in 288 adult male Sprague-Dawley strain rats. The effect of differing initial force magnitudes on phosphatase changes was also examined. Phosphatase measures were obtained by colorimetric assays. Analysis of variance (ANOVA) procedures were performed to examine for differences within groups across time and among groups at each time Point. Data from treated animals were combined, adjusted for control values, and examined across time to permit comparisons with previously described histomorphometric changes. Both the serum and the bone phosphatase data clearly support previous histomorphometric observations that bone turnover, characterized by periods of activation, resorption, and formation, occurs during orthodontic tooth movement; serum data suggested differing force magnitudes may alter the timing of these bone turnover events.

Keywords: Orthodontic Force, Tooth Movements, Periodontium, Alkaline Phosphatase, Osteoblasts.

Introduction

Bone turnover during orthodontic tooth movement has typically been described as a balanced process, characterized by continual bone deposition on the tension side and continual bone resorption at sites of pressure.[1] However, recent histom0rphometric data suggest that this process may be more complex than this classical paradigm. During orthodontic tooth movement in a rodent model, an early wave Of resorption (3 to 5 days), followed by its reversal (5 to 7 days), and a late wave of formation (7 to 14 days), has been described

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in total alveolar tissue, as well as on both the pressure and the tension sides of the alveolar wall. Resorbing cells, such as osteoclasts and macrophages, have been shown to have high acid phosphatase activities; bone forming cells (i.e., osteoblasts) to have alkaline phosphatase activities.[2] In addition, osteoclasts have been demonstrated to have enhanced tartrate-resistant acid phosphatase (TRAP) activities. Changes in acid and alkaline phosphatase in serum and bone have been used as markers to monitor bone changes in several diseases.[3]

Phosphatase changes have been described in orthodontically treated tissues.[4] With descriptive histochemistry and biochemical techniques, previous investigators have reported increased acid phosphatase and decreased alkaline phosphatase activities on the pressure side and increased acid and alkaline phosphatase activities on the tension side of orthodontically treated teeth up to 7 days after appliance activation in the rat.[5] As no description of changes past 7 days was made, it is likely that the reversal in activity of these enzymes was missed in these studies in light of recent histo-morphometric data.[6]

Recent data have also demonstrated that initial force magnitudes of 40 and 60 gm yielded identical tooth movement curves in this rodent model; these movements, however, were greater at all time points than that produced by an initial force of 20 gm.[7] Less inhibition in alkaline phosphatase activity during the first 7 days of rodent tooth movement has been associated with lighter forces in one report, although no quantitative data were presented.[8] Changes in phosphatase activities as a function of differing initial forces over the entire tooth movement cycle have not previously been reported.[9] The purpose of this study was to examine the time course of phosphatase changes during an entire tooth movement cycle, in both the serum and the alveolar bone.[10]

Materials and Method

The data were obtained from 288 adult male Sprague Dawley strain rats (180 to 200 days old). These were shipped by air freight and acclimatized for at least 2 weeks under experimental conditions including being housed in plastic cages, fed a diet of ground laboratory chow and distilled water ad libitum, and maintained on a standard 12-hour light/dark cycle. All animal manipulations, including euthanasia, were done at the same time of day.

After achieving anesthesia, the chest cavity was opened, and the great vessels of the heart cut. Blood was drawn from the chest cavity with 20 ml syringes without needles attached. The blood was stored in test tubes at room temperature to permit clotting. The samples were then centrifuged at 700g for 20 minutes, and the serum was retrieved.

To ensure adequate sample for analyses of the bone enzymes, supernatants obtained from the hemimaxillae of two animals were pooled, resulting in a sample size of six for each time point. For the serum analyses, blood from six randomly selected animals at each time point were analyzed.

Acid phosphatase, TRAP, alkaline phosphatase, and protein were assayed by using commercially available quantitative, colorimetrie kits. The determination of alkaline phosphatase used 2-amino-2-methyl-l-propanol (AMP) (1.5 mol/L, pH 10.3 at 25 ~ C) buffer and involves a 15-minute incubation. The assays are based on the hydrolysis of p-nitrophenol phosphate by the enzymes. The phosphatase readings were performed at 414 nm with a Titertek Multiskan MC, the protein colorimetric assays with a bovine serum albumin standard, and the Coomassie Blue dye method at 595 nm with an Ultrospec 4050 spectrophotometer. Serum phosphatase values were expressed in Sigma units, and

the bone values were calculated as Sigma units per microgram of protein.

Data Analysis

Analysis of variance (ANOVA) procedures were performed to examine for differences within groups (control, 20 gm, 40 gm, and 60 gm) across time and at each time point among groups for each enzyme. Scheffe pairwise comparisons were performed when ANOVA indicated that differences existed ($p < 0.05$). 20 In addition, data from the three force groups for each enzyme were combined, after adjusting for day-specific mean control values, and examined across time by ANOVA and Scheffe comparisons.

Results

Acid, TRAP, and alkaline phosphatase changes in serum and alveolar bone clearly demonstrated that bone turnover is not balanced in the short-term during orthodontic tooth movement. Instead, there seems to be an early period characterized by a preponderance of bone resorption followed by a later period when bone formation is primary.[11] This finding confirms earlier reports of bone turnover characterized by tandem periods of activation, resorption, reversal, and formation occurring after orthodontic force application with histomorphometry 16 and of a transient reduction in bone density in a similar animal model.[12] Differences within groups across time were more obvious in the serum data than in the bone derived enzyme data (Tables I and ll) because the latter had greater variability.[13] This variability might be reduced in future studies by increasing sample size and not pooling the maxillae from two animals. In retrospect, the pooling of hemimaxillae from two animals to create one sample for analysis was unnecessary, as the methods were sufficiently sensitive for the analysis of one hemimaxilla.[14] To examine further for differences across-time. in the bone derived enzymes, the data from all force groups were combined.[15] This was also done with the serum enzyme data for discussion and comparison.[16] The early peak in serum acid phosphatase and TRAP preceded the peak in bone, as well as the peak in several resorptive parameters measured histomorphometrically.[17] This early serum peak occurred during the induction (activation) phase of the bone turnover cycle, during which no bone changes could be quantified histologically.[18] The serum peak fell off early (day 3) and remained depressed until the end of the observation period.[19] The peak in bone acid phasphatase and TRAP activities lasted longer (from days 3 to 7), and reversed between days 7 and 10, remaining depressed at 10 and 14 days.[20] Histomorphometric parameters reflecting resorption followed similar kinetics, reversing between 5 and 7 days. The finding that the serum peak in these enzyme activities preceded their appearance in bone suggests that osteoclasts or preosteoclasts may be detectible during their migration to the treated PDL, and that these data may have some predictive value.[21]

There was a peak in alkaline phosphatase activity in both the serum and alveolar bone at day 7, paralleling the peak in histomorphometric parameters reflecting formation that occurred between 7 and 10 days.[22] However, alkaline phosphatase in serum and bone fell off by day 10 in sharp contrast to the histomorphometric data depicting formation.[44] In addition, a second, but significant, late peak at day 14 was observed in the serum, but not the bone.[23] That the timing of the serum alkaline phosphatase changes reflected those in the bone confirms previous observations that the osteoblasts are present locally without a requirement for significant blood-borne migration.[24]

It is important to consider that the individual effects of chemokines on bone cells

are usually investigated in highly controlled systems (i.e., in vitro or in knockout mouse).[25] When interpreting in vivo data, their putative function of chemokines must be estimated in view of the presence of other cytokines, growth factors and hormones,[43] which can also modulate osteoblast metabolism in a number of ways until the establishment of an overall outcome.[26] In fact, we previously demonstrate an association between the levels of IL-10 and the bone remodeling markers COL-I and OCN [5], suggesting that cytokines, and not chemokines, may play a major role over osteoblasts under orthodontic force.[27] However, further studies are required to evaluate the putative role of chemokines in osteoblast function throughout tooth movement.[28]

Osteoblasts can be an important source of chemokines during orthodontic tooth movement.[29] Chemokine production by osteoblasts can be induced by mechanical stress, inflammatory mediators and even by dentin proteins.[30] The osteoblast derived chemokines include MCP-1 and SDF-1, whose effects on bone cells were previously discussed. Osteoblasts are also able to produce chemokines (such as KC/CXCL1, LIX/CXCL5, CINC-1/CXCL1 and BCA-1/CXCL13) involved in the recruitment of different leukocyte subsets,[31] suggesting an interesting role of osteoblasts in development of inflammatory reaction in periodontal ligament environment after force application.[32] In fact, several immunocompetent cells are recruited to periodontal ligament during experimental orthodontic tooth movement, and in their turn can modulate both osteoblast and osteoclast metabolism.[33] However, the putative role of chemokine-mediated leukocyte migration to periodontium in the outcome of tissue response to orthodontic force remains unknown.[34]

The initially applied forces (20, 40, and 60 gm) used in this animal model might seem excessive to the clinical orthodontist.[35] Histomorphometric and biochemical studies of bone turnover should be performed using lower sustained forces;[36] however, such studies would require the use of coils not commercially available today.[37] By using the lightest coils available, the 20 gm initial force was likely deactivated within the first several days after activation.[38] Although the rate of force decay and average force were not known, it is doubtful that any of the forces applied remained constant during the 14 days of observation.[39] Unlike methods used in previous studies (for example, the use of latex elastics foi'cedinterproximally), this animal model at least permitted the application of well described, measured initial forces.[40] Although it is-dangerous to extrapolate force levels from species to species and impossible to determine what force level is most physiologic in rodents (or human beings),[41] this article and previous articles, which have reported on findings with this model, demonstrated tooth movement kinetics and biologic responses similar to that generally reported with other models, including human models.[42]

Conclusion

The data support the following conclusions:

1. Changes in serum and alveolar bone phosphatases reflect bone turnover in orthodontically treated tissues, supporting previous histomorphometric findings that bone turnover in treated tissues is not balanced in the short-term, but is characterized by tandem periods of activation, resorption, reversal, and formation.
2. The timing of bone turnover events (activation, resorption, reversal and formation) may be altered by differing force magnitudes.

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