ORIGINAL ARTICLE

Lactate dehydrogenase activity in gingival crevicular fluid during orthodontic treatment

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During orthodontic treatment, the early response of periodontal tissues to mechanical stress involves several metabolic changes that allow tooth movement. Many studies have evaluated such modifications by analysis of various host metabolites released into the gingival crevicular fluid (GCF). This study used a cross-sectional design to examine the lactate dehydrogenase (LDH) activity in GCF to assess whether GCF LDH can be proposed as a sensitive marker for periodontal tissue modifications during orthodontic tooth movement. Thirty-seven subjects, 16 males and 21 females (mean age 18.7 years, range 14.0 to 26.7 years), participated in this study. Each subject underwent a session of professional oral hygiene and received oral hygiene instructions; 2 weeks later, a fixed orthodontic appliance was placed on the maxillary arch. A randomly selected maxillary canine was considered as the test tooth, and its antagonist, which had no appliance, was used as the control tooth. From 2 to 12 weeks after orthodontic appliance placement, GCF was harvested from both experimental teeth at the mesiobuccal angle, for GCF volume and LDH activity determinations. Clinical monitoring consisted of recording supragingival plaque presence, bleeding on probing, and probing depth at the same collection sites. The results showed that no differences in clinical conditions and GCF volume occurred between the experimental teeth. On the contrary, GCF LDH activity in the test teeth was significantly greater than that of the control teeth ($P < .01$). Moreover, no differences were found in the enzymatic activity between the sexes by experimental tooth, and no significant correlation was present between GCF LDH activity and patients' ages within experimental teeth. Our enzymatic results initially indicated a possible role of GCF LDH during the early phases of orthodontic treatment and therefore warrant further study as a possible diagnostic tool for tissue response during orthodontic treatment. (Am J Orthod Dentofacial Orthop 2003;124:206-11)

Over the past 35 years, several studies have tried to explain the biomechanical[1] and the biological[2-4] phenomena that allow tooth movement caused by an orthodontic appliance. Studies have evaluated the hard and soft tissue responses during orthodontic treatment in animal[3,5,6] and human[7,8] models. Most have focused on bone metabolism[3,5] or periodontal ligament changes[6,9-11] during tooth movement. Bone metabolism changes seem to be characterized by a combination of tissue resorption and deposition in both the compression and tension periodontal sites.[3,12] Moreover, it has been shown that hyalinization can occur in the most compressed areas of the periodontal ligament after application of orthodontic force on teeth.[9-11] This hyaline zone has also been described as an area of focal aseptic necrosis.[9-11]

To better describe the biological responses to orthodontic force in humans, noninvasive analyses of various cell mediators or enzymes in the gingival crevicular fluid (GCF) have also been performed.[4,13-16] In the GCF, Uematsu et al[13] found several cell mediators, such as interleukin1β (IL1-β), interleukin 6, tumor necrosis factor-α (TNF-α), epidermal growth factor, and β_2 microglobulin, significantly elevated in teeth undergoing orthodontic forces, compared with untreated controls. Through such GCF analyses of orthodontically treated teeth, similar results were found by Grieve et al[14] for prostaglandin E and IL1-β and Lowney et al[4] for TNF-α. Finally, Perinetti et al have reported significant elevations in alkaline phosphatase[15] and aspartate aminotransferase[16] activities in the GCF during the first month of orthodontic treatment. All these studies demonstrate that several processes, eg inflammation, can occur in the periodontal tissues

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surrounding the mechanically stressed teeth. These processes seem to be responsible for the consequent tissue remodeling that allows tooth movement.[4,13-16]

Lactate dehydrogenase (LDH), an enzyme normally limited to the cytoplasm of cells, is only released extracellularly after cell death.[17] Previous studies have demonstrated that the activity of LDH in GCF is significantly correlated with gingival inflammation[18] and tissue destruction from periodontitis.[19] Therefore, it has been proposed that LDH activity in the GCF is a potential marker for monitoring periodontal metabolism.[19]

With the consideration that LDH is an index of tissue destruction,[17,19] and that, during orthodontic tooth movement, phenomena such as cell necrosis, have been described in the periodontal ligament,[9-11] an increase in LDH activity in the GCF can be hypothesized. However, to date, no studies have investigated the possible role of GCF LDH in the early events that lead to tooth movement during orthodontic treatment. This study was therefore designed as a cross-sectional assessment to determine whether GCF LDH is a sensitive marker for periodontal tissue changes during the early phases of such treatment. If so, its use could be an objective tool for monitoring tooth movement in clinical practice, as has been proposed for other host cell mediators and enzymes.[4,13-16]

MATERIAL AND METHODS

Thirty-seven subjects, 16 males (mean age 18.0 ± 3.5 years) and 21 females (mean age 18.4 ± 3.8 years), between 14.0 and 26.7 years (overall mean age 18.7 years) participated in this study. They needed fixed orthodontic treatment because of misaligned and crowded teeth in the maxillary anterior segment. Further inclusion criteria were (1) good general health, (2) no use of anti-inflammatory drugs in the month before appliance placement, (3) probing depth values not exceeding 3 mm in the whole dentition, (4) no radiographic evidence of periodontal bone loss after radiographic periapical examination, and (5) full-mouth plaque score (FMPS) and full-mouth bleeding score (FMBS) ≤20%. FMPS and FMBS were recorded as the percentages of tooth surfaces with supragingival plaque and of bleeding within 15 seconds after probing with a 20-g controlled-force probe (Vivacare TPS Probe, Vivadent, Schaan, Lichtenstein), respectively. Two weeks before appliance placement, all subjects underwent a session of accurate supra- and subgingival ultrasonic scaling, and received oral hygiene instructions about the correct use of a toothbrush and dental floss; the use of an interdental brush was also suggested to properly clean under the orthodontic archwire. Moreover, from appliance placement to GCF sampling, the oral hygiene instructions were further reinforced with all subjects, who were not allowed to take any anti-inflammatory drugs, which could have affected the results.[14] Informed consent was obtained from the patients, or the parents of those under 18 years of age, before the study, and the protocol was reviewed and approved by the Ethical Committee of the G. D'Annunzio University Medical Faculty.

Orthodontic brackets (MBT, 3M Unitek, Monrovia, Calif) were placed on the buccal surfaces of the maxillary teeth, including the incisors, canines, and premolars. Bands were also placed on the first molars. An active 0.016-in circular cross-sectional, nickel-titanium orthodontic wire (American Orthodontics, Sheboygan, Wis) was then mounted on this orthodontic appliance. During the 2 to 12 weeks after appliance placement, patients underwent GCF sampling. In each patient, a randomly selected maxillary canine having the fixed orthodontic appliance was used as the test tooth, and its antagonist, with no appliance, was chosen as the control tooth. The GCF was sampled once for each patient from the mesiobuccal aspect of each experimental tooth.

With each sampling site, the clinical examination consisted of recording the presence of supragingival plaque (PL+), assessed by visual criteria; gingival bleeding within 15 seconds after probing (BOP+) with a 20-g controlled-force probe; and probing depth (PD). The same operator (C.C.) collected all clinical data. Contamination of the GCF samples was minimized by recording the PL+ before carefully cleaning the tooth with cotton pellets, collecting GCF from the isolated area, and then recording the PD and BOP+, as previously described by Griffiths et al.[20] At the mesiobuccal aspect of the test and control teeth, GCF was collected for the LDH activity assay. Each crevicular site included in this study was isolated with cotton rolls. Before GCF collection, any supragingival plaque was removed with cotton pellets, and a gentle airstream was directed toward the tooth surface for 5 seconds to dry the area.[21] The GCF was collected with #30 standardized sterile paper strips (Inline, Torino, Italy) inserted 1 mm into the gingival crevice and left in situ for 30 seconds. The same operator (G.P.) collected the GCF. After collection, the paper points were immediately transferred to plastic vials and stored at −80°C until analyzed. GCF total volume was determined for each sample as previously described.[21] After orthodontic appliance placement, 12 patients were monitored at 2 weeks, 9 at 3 weeks, 7 at 4 weeks, 2 at 6 weeks, 3 at 8 weeks, 3 at 9 weeks, and 1 at 12 weeks.

The GCF LDH activity was determined spectropho-

tometrically[18] (Model 8453, Hewlett Packard, Waldgrohn, Germany) at a constant 30°C, with less than 0.05°C fluctuation. The paper point was incubated for 5 minutes in a substrate containing 16.2 mmol/L pyruvate, 0.194 mmol/L reduced nicotinamide adenine dinucleotide (NADH), 54.0 mmol/L phosphate buffer (pH 7.5 ± 0.1 at 30°C), in a total volume of 1.0 mL. In the presence of LDH, pyruvate is reduced to L-lactate with the simultaneous oxidation of NADH. The rate of decrease in absorbance at 340 nm, representing the NADH consumed, is directly proportional to LDH activity in the sample. A 1-cm path length was used, and a value of 6.22 was considered as the NADH millimolar absorptivity. Results were first converted into enzyme activity units (1 unit = 1 μmol of NAD+ released per minute at 30°C) and are expressed as total LDH activity (μmol units/L) per sample.

The Statistical Package for Social Sciences program (SPSS, Chicago, Ill) was used to perform the data analysis. Data regarding the PD, GCF volume, and GCF LDH activity are presented as mean ± standard deviation (SD). This continual data set was tested for normality with Shapiro-Wilk test and Q-Q normality plots; equality of variance was also tested with the Levene test and Q-Q normality plots of the residuals. With the exception of the PD and GCF volumes, each data set met the required criteria for using parametric analyses. Clinical data obtained from the corresponding sampling sites were processed as follows: the number of sites of PL+ and BOP+ were considered as dichotomous data, while the PD scores were considered as ordinal data; between the groups, McNemar and Wilcoxon signed rank tests assessed the significance of differences in PL+ and BOP+, and PD, respectively. Within each group, differences in the patients' ages were assessed by sex with an unpaired *t* test. Furthermore, the significance of the differences in clinical conditions between the sexes by experimental tooth were evaluated with the Fisher exact test for PL+ and BOP+, and the Mann-Whitney *U* test for PD. The significance of differences in both the overall GCF volume and LDH activity between the groups was evaluated by performing Wilcoxon signed rank and paired *t* tests, respectively. Moreover, for the GCF LDH activity, an unpaired *t* test was used to assess the significance of the differences between the sexes by experimental tooth. Finally, a correlation matrix, using the Pearson correlation coefficient, was computed to evaluate the strengths of the straight-line relationship between the enzymatic activity and patients' ages by group. A probability of $P < .05$ was accepted for rejection of the null hypothesis.

RESULTS

In the study population, no significant difference was found between the sexes for mean age ($P > .5$).

In the test teeth, the number of sites PL+ and BOP+ were 10 and 7, respectively, while the PD was 1.6 ± 0.7 mm. In the control teeth, the number of sites PL+ and BOP+ were 8 and 4, respectively, and the PD was 1.4 ± 0.6 mm. The differences between groups for each variable are not statistically significant. No statistically significant differences were recorded for PL+, BOP+, and PD between sexes by experimental tooth (all comparisons at $P > .1$; data not shown).

The GCF volume in microliters was 0.16 ± 0.09 in the TTs and 0.14 ± 0.06 in the CTs, with no statistically significant difference between the experimental teeth ($P > .1$), even when processed by sex ($P > .1$ and $P > .5$ within the test and control teeth, respectively; data not shown).

The GCF LDH activity in μmol units/L was 365 ± 179 in the test teeth and 117 ± 43 in the control teeth. The difference between experimental teeth is highly significant ($P < .01$). However, no significant differences were found in this enzymatic activity when compared by sex in each experimental tooth ($P > .5$ for both the test and control teeth; data not shown).

Finally, the correlation matrix shows no significant straight-line relationship between GCF LDH activity and patients' ages for both the test ($P > .5$) and control teeth ($P > .5$; data not shown).

DISCUSSION

Our controlled, cross-sectional study has evaluated GCF changes during the early phases of orthodontic treatment from an enzymatic point of view. Although no differences were detected in the gingival conditions and GCF volumes of the samples, the GCF LDH activity levels were significantly greater in teeth undergoing orthodontic forces, as compared with untreated controls. These levels were also not influenced by sex, as the intragroup analyses demonstrate. The correlation matrix shows that these enzymatic-activity increases are also irrespective of patients' ages for both experimental teeth.

The biomechanic principles of tooth movement during orthodontic treatment have been extensively described.[22] These are supported by several studies that have evaluated periodontal changes incident to orthodontic tooth movement.[2-16] Both animal[3,12] and human[14-16] models have been used, as well as cross-sectional[23,24] and longitudinal study designs.[13,14] Hence, it is well known that when a force is applied to a tooth, the periodontal tissues undergo either tension

or compression stress, depending on the tooth movement.[2,9]

LDH is a functionally related intracellular, cytoplasmic enzyme that is released into the extracellular environment upon cell death.[17] Its extracellular presence is related to cell necrosis and tissue breakdown.[17] For this reason, LDH levels in the blood are monitored as a diagnostic index of tissue destruction during acute myocardial infarction or chronic liver disease.[17] GCF LDH activity levels have also been positively correlated with tissue inflammation during gingivitis[18] and tissue destruction caused by periodontitis in humans.[19,25] In the latter studies, the authors observed that successful periodontal treatment could significantly reduce GCF LDH activity levels.[19,25] A further study has correlated the GCF LDH activity with the PD.[26] Although several studies have evaluated LDH activity changes in different periodontal conditions, evidence of a possible role of GCF LDH during orthodontic treatment is still lacking.[6,18,27]

Gingival modifications incident to tooth movement have been reported in both histological and ultrastructural analyses.[28] Clinically evident changes have also been detected.[28] The subjects in the present study showed, however, similar gingival conditions for the test and control teeth. This is probably due to the oral hygiene instructions given to each participant before treatment and followed by further reinforcements about gingival health throughout the treatment. It has been reported that good maintenance of clinical conditions is possible despite orthodontic appliance placement.[29,30] However, other studies have not shown similar results in terms of gingival conditions.[31] In our study, we monitored the canines of all patients, because these teeth are accessible and easily cleaned. It has also been reported that plaque accumulation depends on the site; more plaque accumulates in the anterior area than in the posterior area.[32] Moreover, although the PD difference was not statistically significant between the groups, a slightly greater value was recorded for the test teeth, as also seen for PL+ and BOP+. This minimal increase can presumably be correlated with the gingival enlargement during orthodontic treatment found in other studies.[31] However, because these differences are minimal and not statistically significant, and the data regarding the GCF volume from the experimental teeth are very similar, it can be assumed that PL, BOP, and PD have not influenced the GCF LDH activity[18] recorded for the TTs.

The GCF volume from the test teeth was similar to that from the control teeth. Previous studies have described a significant positive correlation between GCF volume and periodontal inflammation.[33] The lack of inflammation reported here supports the finding of similar GCF volume scores found in the experimental teeth. In previous studies,[15,16] we reported that GCF volume is not influenced by the tooth movement but is influenced instead by the inflammation of the periodontal tissue. Conversely, Samuels et al[7] have reported that, although no gingival health changes occur, the GCF volume can significantly increase during orthodontic treatment. A possible explanation of this difference from our results might derive from the different methods used for GCF collection; Samuels et al[7] used microcapillary tubes kept in situ for 15 minutes, but we sampled the GCF by adsorbent paper points kept is situ for 30 seconds. A further possible explanation for our results is that the small volumes recorded for the GCF sample in the present study could have led to relatively large errors in their determinations.[27]

It has been shown that levels of biochemical markers in the GCF, such as calprotectin, might depend on different collection sites.[34] For this reason, in the present study, the canines were used as both the test and control teeth. The GCF LDH activity was significantly greater in the test teeth compared with the control teeth; however, the enzymatic activity increases were not related to age or sex (data not shown). This increase is not surprising because, during orthodontic treatment, periodontal changes have been shown such as tissue resorption or destruction.[3,6] In the periodontal ligament, a hyalinization of the most compressed area induced by compressive forces has been reported.[9-11] This hyaline zone is described as an area of focal aseptic necrosis[9-11] that resists degradation, persists in the pressure zone, and depends on the magnitude of the force.[1] In this region, as observed by light microscopy, all cellular structures are missing,[6] but a variety of cell fragments have been shown at the ultrastructural level.[9] Moreover, in a previous study[6] conducted on a rat model, cytoplasmic enzymes such as LDH were found in this extracellular environment. The significant increase in the GCF LDH activity in the test teeth agrees with these reports, although the present study does not distinguish between compression and tension sites. The finding of an elevation of GCF LDH activity during periodontal metabolism change is supported by the demonstration that GCF is an exudate whose constituents derive from various sources, including microbial dental plaque, host inflammatory cells, host tissue, and serum.[33,35]

A further explanation of the results reported here might derive from observations of periodontal tissue resorption and deposition during orthodontic tooth movement. As initially described by Rygh,[9,10] bone remodeling determined by tooth movement is a continuous process characterized by bone resorption in the

compression sites[9] and bone deposition in the tension sites.[10] However, recent histomorphometric findings[3] have shown that this model is more complex than that described by Rygh. Both bone resorption and deposition can be present in any tension site, as well as in any site of compression.[3] This model is based on an initially unbalanced process, in which resorption is greater than deposition, and a later more balanced relationship between these phenomena.[3,12] However, these observations were made through stressing the alveolar bone, by tooth movement, in a rat model.[3,12] Furthermore, the authors 3 describe an early phase of bone resorption (3 to 5 days), its reversal (5 to 7 days), and a late phase of bone deposition (7 to 14 days) in both tension and pressure sites of the alveolar wall. A similar bone cycling in humans has also been reported.[36,37] In humans, however, this timing seems to be longer than that in the rat alveolar bone. In particular, there is a sudden initial activation phase followed by resorption, which can last from 10 days[37] to 3 weeks.[36] Finally, an 80-day period is characterized by bone deposition.[37] These studies might therefore support our increase in GCF LDH in the test teeth from 2 to 12 weeks of treatment. This GCF enzymatic activity increase might be consequent to the tissue resorption in both the compressed and tensional sites,[3] or even secondary to a possible cell necrosis in the periodontal ligament during the orthodontic treatment.[6,9-11] Although our results are supported by previous studies, longitudinal data are required to further assess this GCF LDH role in the events that lead to tooth movement during orthodontic treatment.

CONCLUSIONS

Our findings of an LDH enzymatic-activity increase in GCF are relevant when it is considered to be associated with clinically undetectable modifications. Thus, within the limitation of this study, GCF LDH can be proposed as a sensitive marker of the periodontal metabolism changes during orthodontic tooth movement.

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