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DOI: 10.2478/aoj-2008-0018

The effects of systemic stress on orthodontic tooth movement

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Objectives: To determine if systemic stress affects the biological reactions occurring during orthodontic tooth movement.
Methods: Four groups of male 10 week-old Wistar rats were used. Group A animals (N=10) were restrained for one hour per day for 40 days; Group B animals (N=10) were restrained for one hour per day for three days; Group C (N=10) and Group D (N=8) animals were unrestrained. The upper left first molars in the rats in Groups A (long-term stress), B (short-term stress) and C (control) were moved mesially during the last 14 days of the experiment. The animals in Group D (N=8) were used for body weight and hormonal dosage comparisons only. They were not subjected to any stress and did not have appliances fitted. All animals were killed at 18 weeks of age and blood collected for measurement of plasma corticosterone. Tooth movement was measured with an electronic caliper. The right and left hemi-maxillae of five rats from each group were removed and the number of tartrate-resistant acid phosphatase (TRAP) positive cells, defined as osteoclasts, adjacent to the mesial roots of the upper first molars counted. The contralateral side in each animal served as the control (split-mouth design).
Results: Corticosterone levels were significantly higher in the stressed groups (Groups A and B) than in the control group (Group C). Tooth movement was significantly greater in Group A (long-term stress) compared with Group B (short-term stress) and Group C (control), which did not differ from each other. There were significantly more osteoclasts in the long-term stress group than in the short-term stress and control groups.
Conclusion: Persistent systemic stress increases bone resorption during orthodontic tooth movement. Systemic stress may affect the rate of tooth movement during orthodontic treatment.
(Aust Orthod J 2008; 24: 121–128)

Received for publication: May 2008
Accepted: October 2008

Introduction

Psychological stressors can enhance bone resorption induced by periodontal disease.[1–3] However, the role of systemic stress on bone remodelling during orthodontic movement is not well understood. Orthodontic patients may be affected by psychological stress-related disorders such as depression, anorexia nervosa, obsessive–compulsive disorder, panic anxiety and chronic alcoholism. The high prevalence of these disorders and increasing evidence of the role of systemic factors on orthodontic treatment emphasise the need for a better understanding of the influence of systemic stress on the tissue reactions during orthodontic tooth movement.[4,5]

The term 'stress' defines the psychophysiological reactions of the body to a variety of emotional and/or physical stimuli that threaten homeostasis. The neuroendocrine substances secreted in these reactions

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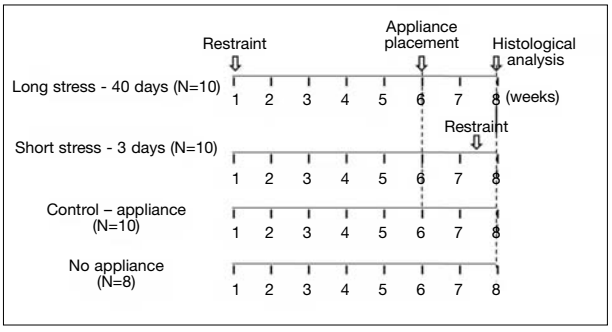


Figure 1. Study design. Group A was restrained intermittently over 40 days and received the orthodontic force (long-term stress group).
Group B received the orthodontic force and was restrained intermittently for three days (short-term stress group).
Group C had an orthodontic appliance only (control group).
Group D was not restrained and had no appliance (no appliance group).
The latter group was used for body weight and hormonal level comparisons.

are involved in every organ system and in many physiologic, cellular and molecular networks.[6] The central components of the stress system are located in the hypothalamus and the brainstem, and include the corticotropin-releasing hormone (CRH) and the locus ceruleus-norepinephrine/autonomic sympathetic nervous systems.[7] The peripheral limbs of the stress system are the hypothalamic–pituitary–adrenal (HPA) axis, together with the efferent sympathetic/adrenomedullary system and components of the parasympathetic system.[8] The central CRH and norepinephrine systems, together with peripheral secretion of large amounts of glucocorticoids and catecholamines, affect virtually every cell in the body.[9]

In orthodontic therapy, the application of controlled mechanical forces to teeth initiates complex biochemical interactions leading to bone remodelling,[10] which can be regulated either by local factors such as the applied forces, or by systemic factors, such as drugs, hormones and vitamins.[4,5,11] A growing number of studies suggest that sympathetic nerves modulate inflammation and bone remodelling in vivo.[12–14] Osteoblasts and osteoclasts are well-equipped with adrenergic and glucocorticoid receptors,[15,16] indicating that these cells are influenced by sympathetic neurotransmitters. It has also been reported that bone resorption increased following sympathectomy,[17,18] which supports the hypothesis that orthodontic tooth movement is influenced by the body's response to systemic stress.

Although it is well recognised that synthetic glucocorticoids influence bone metabolism and tooth movement,[19–22] little is known about the role of endogenous glucocorticoids on orthodontic tooth movement. The synthetic derivative of cortisol differs from the natural hormone in strength, and only mimics the dynamics of a normal adrenocortical response during stress.[23] Furthermore, the stress response involves an orchestrated interplay of several neurotransmitter systems, not only the glucocorticoids.[24] Therefore, the present study was designed to determine if systemic stress affected the number of osteoclasts and the rate of tooth displacement during orthodontic tooth movement.

Materials and methods

Animals

Ten week-old male Wistar rats, weighing 275 g on average at the beginning of experiment, were obtained from Centro Multidisciplinar de Investigação Biológica-Cemib, Unicamp, Campinas, Brazil. The rats were housed in groups of five and maintained in a temperature controlled room (23 ± 1°C) with a 12/12 light–dark cycle. Ground pellets and water were available ad libitum. Throughout the experiment all animals were weighed once a week. The study was conducted in accordance with the ethical guidelines for investigations of experimental pain in conscious animals.[25] The research was approved by the institutional ethics committee in animal experimentation, according to the Brazilian College of Experimentation Guidelines.

The animals were distributed into four groups as follows: Group A (N=10) animals were submitted to long-term stress before and during orthodontic tooth movement; Group B (N=10) animals were submitted to short-term stress during orthodontic tooth movement; Group C (N=10) animals had an active orthodontic appliance inserted, but were not subjected to any stress. Group D (N=8) animals were used for body weight and hormonal dosage comparisons only. They were not subjected to any stress and did not have appliances fitted. The study design is shown in Figure 1.

Stress exposure

The animals in Group A (long-term stress) were restrained for 1 hour daily, 5 days per week for 40 days. The animals in Group B (short-term stress) were restrained for 1 hour daily for 3 days (days 11, 12 and 13 after appliance placement). The stress

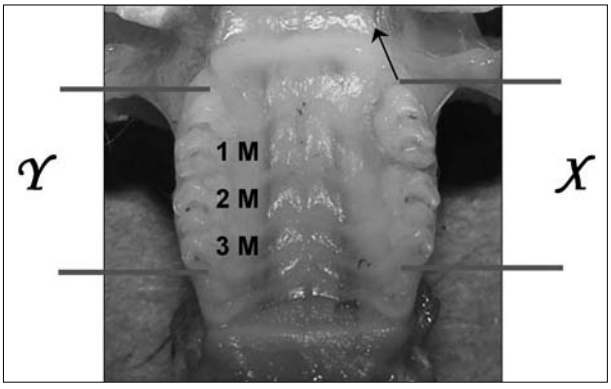


Figure 2. Measurements used to estimate the rate of tooth movement. The distances between the mesial of the first molars (1M) and the distal of the third molars (3M) were measured on the treated side (X) and the untreated side (Y). Tooth movement = X minus Y. The arrow indicates the direction of the orthodontic force applied for 14 days.

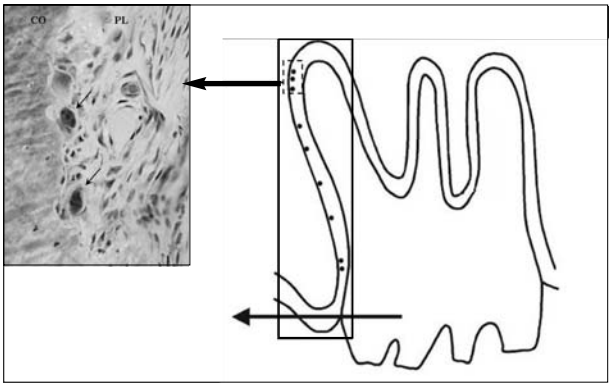


Figure 3. The area (large rectangle) where tartrate-resistant acid phosphatase (TRAP)-positive cells were counted. The arrow indicates the direction of orthodontic force applied for 14 days. The smaller dashed area shows TRAP-positive multinucleated osteoclasts (20x magnification) on the experimental side in a rat submitted to long-term stress. CO: 'compression' side alveolar bone; PL: periodontal ligament.

procedure in Group A consisted in 5 days of stress followed by 2 days of rest until 60 days: the orthodontic force was applied in the last 14 days. These stress protocols follow the design used by Gameiro et al.[26,27] Restraint was carried out by placing the animal in an adjustable plastic restraint tube for 1 hour. The size of the tube was adjusted with velcro straps to suit each rat and there was a 1 cm hole for breathing. Group C (control group) and Group D animals were not restrained.

Hormonal assays

The rats were decapitated immediately after the last stress session and whole blood collected. The time interval between the stress procedure and sacrifice was maintained at 30 seconds for all groups. Plasma corticosterone levels were determined by radioimmunoassay after plasma extraction using ethanol as previously described.[28]

Appliance placement and measurement of tooth movement

The appliance used has been previously described by Gameiro et al.[29] The animals were anaesthetised with xylazine (10 mg/kg) and maintained with ketamine (50 mg/kg). A closed coil nickel-titanium (NiTi) spring (Sentalloy, GAC, Central Islip, New York), calibrated to provide a force of 50 g, was ligated to the upper left first molar and connected to hook soldered on an orthodontic band cement cemented on the incisors. The hook was soldered near the gingival margin to direct the force close to the centre of resistance of the molar. Previous studies have demonstrated that a force of 40–60 g resulted in substantial molar tooth movement in rats.[30,31] NiTi springs were used to provide a relatively constant force over the course of the experiment. To limit the influence of inter-animal variation a split-mouth design was used and the untreated contralateral side served as the intra-animal control. After 14 days of tooth movement the rats were decapitated, whole blood collected and the maxillae excised. The distances between the mesial surfaces of the first molars and the distal surfaces of the third molars were measured bilaterally with an electronic caliper (Digimatic-Mitutoyo, Telford, UK) under a dental operating microscope (D.F. Vasconcellos S.A., São Paulo, SP, Brazil) at 16x magnification. Tooth movement was estimated by subtracting the mean of repeated measurements of the untreated sides from the mean of repeated measurements of the treated sides, as described by Hong et al. (Figure 2).[32] The errors were estimated from two measurements performed on 20 randomly selected animals using Dahlberg's equation.[33] The error in measurement was 0.02 mm and was thus considered to be of no further importance.

Tartrate-resistant acid phosphatase histochemistry

The right and left hemi-maxillae of five rats from each group were processed for tartrate-resistant acid phosphatase (TRAP) staining.[34] From each specimen

three 5 μm sagittal sections were taken at 50 μm intervals. The first section passed through the pulp of the mesial root of the upper first molar. The slides were counterstained with Harris' haematoxylin and eosin for 7 minutes. Cover slips were mounted with Entellan and the slides examined with a light microscope (Leica Microsystems, Wetzlar, Germany).

In each section the osteoclasts were counted at the alveolar bone surface ('compression' side) adjacent to the entire mesial root. Cells were considered to be osteoclasts if they were multinucleated, TRAP positive and located on or close to the surface of the bone (Figure 3). The total number of TRAP positive cells in the three sections taken from each tooth in each animal was recorded. The mesial root was chosen because it is the largest root on the upper first molar, it is approximately in the same plane as the applied force and it is the root most commonly used in tooth movement studies.[22,29]

Cell counting was performed manually in a blinded manner, and a reproducibility error of less than 10 per cent was established by re-counting 20 randomly selected images. These counts were then compared with the original counts with Dahlberg's equation. The error for the TRAP variable was 0.62 and was thus considered to be of no further importance.

Statistical analyses

Descriptive statistics were calculated for all groups. Comparisons of tooth movement and plasma corticosterone levels were made using the analysis of variance (ANOVA) and Tukey's HSD test. The number of osteoclasts in each group was compared using split-plot ANOVA and Tukey's HSD test after square-root transformation to produce normal distributions of the data and to allow parametric analyses. Body weight was analysed by repeated measures ANOVA and Tukey's HSD test. The SAS software (Version 9.1, 2003; SAS Institute Inc., Cary, NC, USA) was used and the significance level was $p < 0.05$.

Results

Animal status and hormonal dosages

All animals with appliances lost weight temporarily (1–2 days), but had an overall gain in body weight over the ensuing weeks ($p < 0.000$; Figure 4). Restraint had no immediate effect on body weight ($p = 0.813$), but there was a significant interaction between weeks and treatment ($p < 0.000$). There was a significant increase in plasma corticosterone levels in the long- and short-term stress groups ($p < 0.000$; Figure 4). There were, however, no statistically significant differences in plasma corticosterone levels between the no-appliance (Group D) and control (Group C) groups, or between the long-term (Group A) and short-term (Group B) stress groups (Figure 4).

Tooth movement

All molars subjected to orthodontic forces had moved mesially and spaces had developed between the first and second molars. The amount of tooth movement was significantly greater in the long-term stress group than in the control and short-term stress groups ($p < 0.005$; Figure 5). The difference between the control and short-term stress group was not significant (Figure 5).

TRAP cells

TRAP-positive cells were detected in all groups. There were significantly more TRAP-positive cells lining the alveolar bone adjacent to the mesial root on the experimental side than on the control side in Groups A, B and C ($p < 0.005$; Figure 6). Comparisons between the experimental sides revealed that there were significantly more TRAP-positive cells in long-term stress group compared with the control and short-term stress groups, which did not differ between each other (Figure 6).

Discussion

In this study, we evaluated the alveolar bone changes during orthodontic tooth movement in rats submitted to long- and short-term stress. We confirmed the efficacy of our model for inducing stress-like hormonal changes by observing that plasma corticosterone levels increased significantly in both the long- and short-term stressed groups (Figure 4). The orthodontic appliance alone did not influence the corticosterone levels after 14 days of tooth movement (Figure 4). Although orthodontic tooth movement is known to cause inflammatory reactions in the periodontium and dental pulp,[10,18] which stimulate release of various biochemical mediators causing the sensation of pain,[35] the orthodontic pain usually peaks at 24 hours after archwire placement and then declines over the following 7 days.[36,37] Thus, orthodontic pain, which could activate the stress system, was

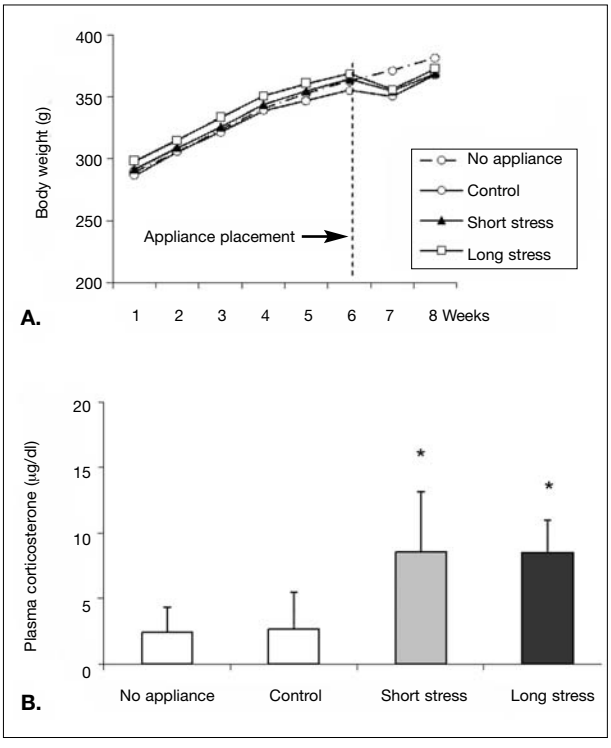


Figure 4. (A) Body weight over the course of 8 weeks, (B) Plasma corticosterone level after the experimental period. Each column represents the mean. Errors bars indicate the SD. Single asterisks indicate significant differences between the stress groups and the control and no appliance groups.

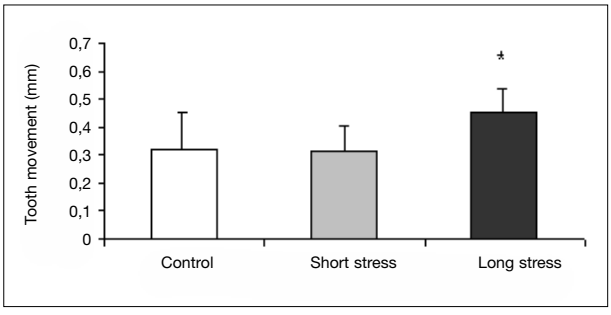


Figure 5. The effects of short- and long-term stress on tooth movement in rats. Each column represents the mean. Error bars indicate the SD. Single asterisk indicates a significant difference between the long-term stress group and the control and short-term stress groups.

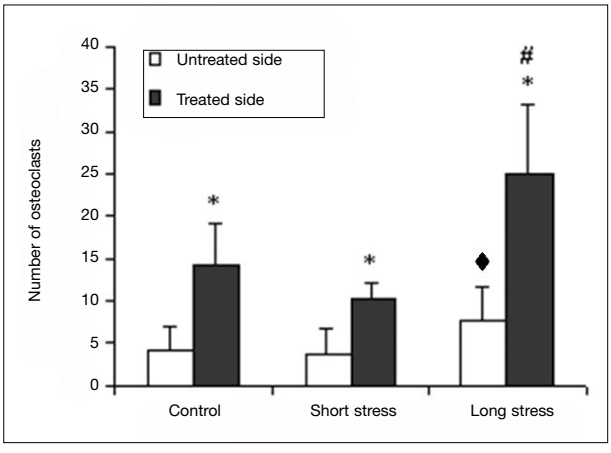


Figure 6. The effects of short- and long-term stress on the number of osteoclasts induced by orthodontic tooth movement in rats. Each column represents the mean. Error bars indicate the SD. The number of osteoclasts on three sections, selected at 10 section intervals were summed for each sample (N = 5/group). White bars: untreated side; black bars: treated side. * Significant differences between untreated vs. treated sides. ◆ Significant difference between untreated sides in the long-term stress group vs. control and short-term stress groups. # Significant difference between treated sides in the long-term stress vs. control and short-term stress groups.

absent after 14 days of tooth movement indicating that the orthodontic appliance was not a stress factor in this experiment.

The present study showed that the amount of tooth movement increased in rats submitted to long-term stress (Figure 5). This indicates that prolonged stress increased the localised bone resorption induced by the active orthodontic appliance, as confirmed by the elevated number of osteoclasts on the experimental side in the long-term stress group. The number of osteoclasts was higher in this group than in the short-term stress and control groups, which did not differ from each other (Figure 6). Although the method we used to measure tooth movement had high reproducibility, it underestimates the number of surfaces undergoing resorption on the first molars and, furthermore, the tensed transeptal fibres would have moved the second and third molars on the experimental sides. Since the aim of the present study was to evaluate the interaction between a systemic factor and a standardised local mechanical perturbation, the underestimation of the amount of tooth movement was considered acceptable. In the short-term stress group, the number of osteoclasts on the experimental side was similar to the control group, and this could explain the absence of a statistically significant difference between the rates of tooth movement in the control and short-term stress groups (Figures 5 and 6). Thus, these results suggest that prolonged activation of the stress system is necessary before systemic stress can be considered capable of influencing orthodontic tooth movement.

The periodontal literature contains numerous studies examining the relationship between psychological stressors and destructive periodontal changes

mediated by the stress system.[1–3] According to these studies, stress alone does not result in alveolar bone loss, but may modulate the pathophysiological processes in periodontal inflammation resulting in accelerated degradation of the periodontal tissues.[1–3] This modulation is thought to be mediated by products of the neuroendocrine systems that are released under stress conditions and influence the function of neutrophils, lymphocytes and macrophages, suppressing the cellular immune response and enhancing likelihood of infection and bone resorption.[38,39] It is well-known that orthodontic tooth movement involves a sterile inflammation to remove bone and eliminate (dissipate) the aggressor, which in this case is represented by the orthodontic force and not by an infection. The present study is the first to demonstrate significant interactions between stress and bone metabolism during orthodontic tooth movement. Our results indicate that restraint stress can increase bone turnover around the untreated and treated teeth, increasing the recruitment and activity of osteoclasts and increased tooth movement (Figures 5 and 6).

Considering that the tooth movement induced by the application of a controlled mechanical force releases various inflammatory cytokines and neuropeptides, such as prostaglandins, leukotriens, substance P, histamine, enkephalin, dopamine, serotonin, glycine, glutamate, gamma-amino butyric acid, calcitonin gene-related peptide (CGRP), vasoactive intestinal polypeptide (VIP), neuropeptide Y (NPY) and endothelial adhesion molecules, future studies should evaluate the roles of these substances on the interactions between systemic stress and orthodontic tooth movement.[5,10,14,18,35,40]

A possible explanation for the increased number of osteoclasts and increased rate of tooth movement in the chronically stressed animals in our study may be increased secretion of parathyroid hormone (PTH). PTH is produced by the parathyroid glands and its function is to regulate serum calcium concentration by concerted effects on the kidneys and bone. PTH increases renal calcium re-absorption and stimulates urinary phosphate excretion. In bone, PTH can induce a rapid release of calcium, but it also mediates longer-term changes by acting directly on osteoblasts and indirectly on osteoclasts. In osteoblasts, PTH affects cellular metabolic activity, gene transcriptional activity and multiple protease secretions. Its effects on osteoclasts occur by the production of receptor activator of nuclear factor kappa B-ligand (RANK-L), a protein that plays a crucial role in osteoclast formation and activity.[41] Thus, the increase in bone turnover induced by PTH could accelerate orthodontic movement.[42,43] Although excessive glucocorticoids are a well recognised cause of reduced intestinal calcium absorption,[44] which represents a physiological stimulus to PTH secretion,[41] little is known about the role of endogenous glucocorticoids in determining serum calcium and PTH secretion. The fact that restraint stress can reduce the serum concentration of calcium in rats,[45] lead us to infer that this could be the case in our experiment, leading to an enhanced PTH secretion, increased osteoclastic activity and accelerated orthodontic tooth movement in the chronically stressed rats. These results need to be re-evaluated and confirmed under other experimental conditions, such as the evaluation of calcium and PTH concentrations in rats subjected to restraint stress. Finally, our findings in rats should be confirmed and validated in humans.

Conclusion

Chronic stress significantly increased osteoclastogenesis and tooth movement in teeth subjected to an orthodontic force. We conclude that systemic stress may interfere with orthodontic treatment.

Acknowledgments

The authors thank Gláucia M. B. Ambrosano for statistical analyses. Thanks are due to Maria Aparecida Santiago Varella and Eliene Aparecida Orsini Narvaes Romani for technical assistance. This work was supported by CAPES and FAPESP, Brazil.

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