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Effect of psychological stress on orthodontic tooth movement in rats

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Abstract

Introduction: The purpose of this study was to investigate the effect of psychological stress on orthodontic tooth movement in Wistar rats.

Materials and methods: Forty-eight female ten-week old Wistar rats with an average weight of 188 ±12 gr were selected and randomly divided into two experimental and control groups. The experimental group received crowded environment-induced and cat odour stresses 4 weeks before spring insertion. On the 29th day in both groups, maxillary incisors were moved by the insertion of springs and exactly after 7 days, 9 rats from each group and after 14 days the remaining rats were sacrificed. Then the mesioincisal distance between maxillary incisors was measured. Afterwards, histological sections were prepared to count osteoclasts under a light microscope. The data on the extent of orthodontic tooth movement and the number of osteoclasts were analyzed by independent sample t-test.

Results: The results indicated that on the 7th day after spring placement the orthodontic tooth movement was significantly higher in the control group compared to the experimental group ($p<0.05$). The number of osteoclasts at a significance level of $\alpha=0.1$ in the control group was higher compared to the experimental group. On the 14th day after spring placement, the orthodontic tooth movement in the control group was significantly higher compared to the experimental group ($p<0.05$). However, there were no significant differences in the number of osteoclasts between the two groups. The rats experienced weight loss in the experimental group ($p<0.05$).

Conclusions: Psychological stress led to a decrease in orthodontic tooth movement and in the number of osteoclasts around the root in the movement direction in rats, but a decrease in osteoclast counts was not parallel with time and demonstrated a nonlinear pattern. In addition, psychological stress led to weight loss in rats.

Key words: *Orthodontic tooth movement, psychological stress, prostaglandin, rat.*

Introduction

When force is applied to a tooth during orthodontic tooth movement, mechanical stress is loaded on the alveolar bone. Alveolar bone and the periodontal ligament (PDL) are compressed on one side, while on the opposite side, the PDL is stretched. Mechanical stress on the stretched PDL induces alveolar bone modeling (surface apposition of bone), while mechanical compression gives rise to bone remodeling (the turnover of bone in small pockets (1,2).

Orthodontic tooth movement is an inflammation-like process, in which inflammatory mediators play an important role. When orthodontic force is applied to a tooth, cells and vessels in the PDL are affected and chemical mediators such as prostaglandin E (PGE), interleukin 1-β, nitric oxide (NO) and tumor necrosis factor (TNF) are released, influencing osseous cells (i.e. osteoblasts and osteoclasts) and therefore contributing to orthodontic tooth movement (3-6).

Various factors and mediators influence orthodontic tooth movement and based on the type of the factor may lead to an increase or a decrease in the rate of tooth movement. An alteration in the blood flow within the PDL is the result of sustained pressure, causing the tooth to shift position within the PDL space, compressing the ligament in some areas while stretching it in others. Blood flow decreases where PDL is compressed and within minutes the first messengers, such as cytokines and prostaglandins, are released and the concentration of second messengers, such as cAMP and cGMP, increases in 4 hours. Neuropeptides such as substance P, vasoactive intestinal polypeptide (VIP), calcitonin gene-related peptide (CGRP) and others act as neurotransmitters in the PDL and establish a link between physical stimulus and the biochemical response (6).

Inflammatory process is modulated by endocrine and neurologic mechanisms and growth hormone, androgens, parathormon (PTH), proteins and CGRP-positive nerves influence modeling and remodeling mechanisms (7,8).

Psychological stress is one of the old warhorses of psychiatry. It has been regarded as a cause of major psychopathology and a contributor to considerable mental anguish. For centuries it has been observed that illness often follows stressful life events (9,10).

Patients, frequently, have functional complaints and high levels of anxiety about their appearance during orthodontic treatment. Sari et al indicated that anxiety level in patients and parents who were about to start orthodontic treatment was high (11). Research studies have reported that the most frequent complaints are impaired speech, impaired swallowing, feeling of oral constraint and lack of confidence in public (12). However, pain in orthodontic tooth movement, duration of treatment and problems with appointments and esthetic problems with appliances are other stressful events in orthodontic treatment. It should be pointed out that stress may originate from other conditions apart from malocclusion and orthodontic treatment.

Stimulation of hypothalamic-pituitary-adernocortical system leads to the elaboration of corticotrophin (ACTH), endorphins and glucocorticoids. Some of these hormones have been reported to have immunopotentiating effect, whereas others are primarily immunosuppressive (9, 10). Cortisol, one of the most important glucocorticoids, is a hormone produced in the adrenal cortex after exposure to psychological stress. It has a major impact on the intermediary metabolism, induces an increased blood sugar concentration and influences fat metabolism. In addition to these endocrinologic effects, cortisol has major anti-inflammatory and immunosuppressive properties, inhibiting formation of lymphocytes, inducing lymphatic tissue hyperplasia, suppressing humoral immune defense and reducing synthesis of some pro-inflammatory cytokines (13).Psychological stress is primarily immunosuppressive (13-15). Cortisol is primarily immunosuppressive, causing leukocyte depletion and retarding its function (primarily T-lymphocytes and monocytes) and decreasing natural killer (NK) cell activity (13). Stress decreases the speed of wound healing by impairing the inflammatory response and reducing IL-1α, IL-6 and IL-8 levels in wound site (16, 17).

Several researchers suggest that emotional stress suppresses immune responses and is a predisposing factor in periodontal disease (9,13). Disruption of the circadian rhythm caused by psychological stress, traveling or irregular sleep patterns may adversely affect the production of osteoblasts (18).

Since orthodontic forces implicate both the neural and immune processes, and regarding the inflammation-like nature of orthodontic tooth movement, it is possible that psychological stress influences orthodontic tooth movement.

The purpose of this study was to evaluate the effect of psychological stress on orthodontic tooth movement in rats, so that it would be possible to gain knowledge on environmental or subsidiary factors involved in the process, facilitating orthodontic tooth movement by eliminating the impeding factors or enhancing positive factors. Therefore, it would be possible to avoid the complications of orthodontic tooth movement and reduce the duration of orthodontic treatment.

Materials and Methods

Forty-eight approximately 10-week-old female Wistar rats with an average weight of 188± 12 gr were selected and randomly divided into two equal control and stress groups.

Female animals were selected because the hypothalam-

ic-pituitary-adrenal axis and sympathicoadrenal system of female rats are more reactive to stressful stimuli compared to male rats (19).

To adapt the rats to the environmental conditions, they were kept in standard 12-hour light and dark intervals at 24˚C for a week. Afterwards, the experimental group received crowded environment-induced (housing density) and cat odour stresses for 4 weeks before the insertion of springs. In the experimental group 12 rats were housed in a cage (30×20×15cm) and were exposed to a cloth impregnated with the smell of a cat for 4 weeks. The cloth was placed in a corner in the cage. In the control group 3 rats were housed in a cage and were exposed to a clean cloth (20- 25). The rats in both groups received drinking water and standard laboratory food ad libitum during the study period.

On the 29th day tooth-moving springs were placed on maxillary incisors in both groups. The rats were monitored during the study and were weighed at the beginning of the study before spring placement and at the end of the study.

Researchers have used different methods to insert springs on rat teeth (5,26- 28). Each of these methods has advantages and disadvantages.

The method used in this study was a combination of different methods with some modifications. Springs were made of 0.35 mm round stainless steel wire (Dentaurum, Germany) and welded on central bands (band material 0.12×4.65 mm, Dentaurum) (Fig. 1).

Prior to spring placement the rats were anesthetized using 85 mg/kg Ketamin in a peritoneal procedure. After making sure of anesthesia depth and fixing the animals, the springs were cemented with glass-inomer cement (Bandtite, American orthodontics, USA) (Fig. 2).

Considering the results of previous studies, a 30-gr force was used to move maxillary incisors in rats (29,30). This amount of force is less than the 90-gr force necessary to open maxillary sutures in rats (31).

All the procedures were carried out according to the standards for the care of laboratory animals and were approved by the Medical Ethics Committee of Tabriz Medical University.

Seven days after spring placement 9 rats and fourteen days after spring placement the remaining rats were sacrificed with an overdose of anesthetic agent. On the 7th day after spring placement, when 9 rats in each group were sacrificed, 9 rats were added to stress cage to maintain housing density. Then the distance between the mesial line angles of maxillary central incisors in both groups was measured using digital calipers, with a measuring accuracy of 0.01 mm. It should be pointed out that measurements were made separately by two experienced individuals at two different times and the means of the measurements were accepted as the mesioincisal distance. The two individuals did not know which rat belonged to which group. Subsequent to measuring the amount of orthodontic movement, premaxillae of rats were removed along with the incisor teeth and placed in 10% formalin for 4 days for fixation so that the number of osteoclasts could subsequently be counted. Then the specimens were placed in 10% nitric acid for 2 days for decalcification. After decalcification, the specimens were rinsed for two hours and again placed in 10% formalin for 3 days for fixation. Finally, the specimens were placed in special molds in a specific direction. The specimens were finally placed in paraffin molds ready to be sectioned. The specimens were sectioned serially by a microtome at 5 µm thicknesses perpendicular to the long axis of the incisor teeth in the bone. Five serial sections were prepared at 5 µm thicknesses below the alveolar crest.

The specimens were stained with Hematoxylin and Eosin. To standardize the area for osteoclastic count, a tangential line was drawn on the histological plate on the upper border of the teeth (toward the nasal cavity)

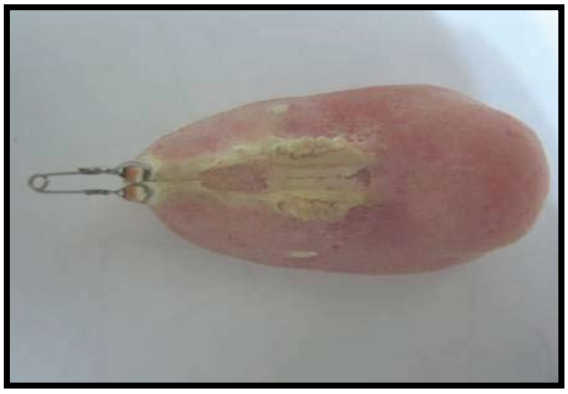

Fig. 1. Premaxilla placed in the acrylic pattern, in which the custom-made spring design is seen.

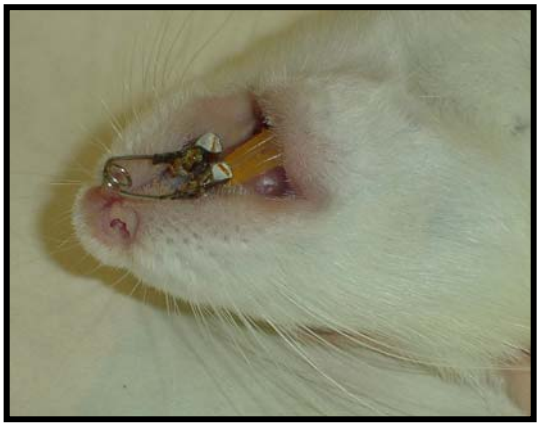

Fig. 2. The spring placed on rat central teeth was fixed with glass-inomer cement. Before cement hardening the space between the teeth was thoroughly cleaned so that the teeth would not stick together.

and perpendicular to the nasal septum. Another line was drawn parallel to that line, 2 mm away from that on the lower side of the teeth. The third line was drawn tangential to the external border of the alveolus and perpendicular to the two above-mentioned lines. The fourth line was drawn 1 mm away from the third line and parallel to it on the nasal septum side. This way we had a 2-mm^2 surface area on the external side of the alveolus for each incisor tooth, which added up to 4 mm^2 of the alveolar surface on both sides for osteoclastic count (Fig. 3).

Therefore, in each rat 5 sections and in each section the distal surfaces of the right and left incisors were evaluated. On the whole, 10 pressure areas with a total surface area of 20 mm^2 were measured and evaluated in each rat. Osteoclastic count was carried out only on the internal surface of the alveolus, which was under pressure, and resorption lacunae with multinuclear cells were considered to be osteoclasts (Fig. 4). Two histologists counted the osteoclasts twice at different times under a light microscope (Olympus, Japan) in a 1-mm^2 surface area. None of the histologists had any information regarding which specimen belonged to which group. Then the means of the counts were accepted as the number of osteoclasts in that histologic section.

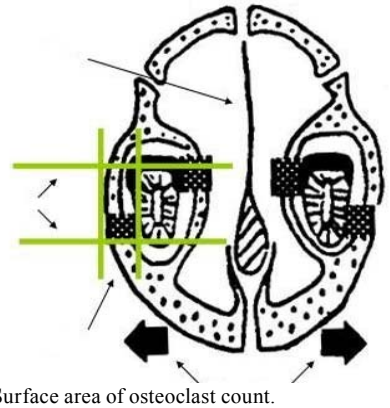

Fig. 3. Surface area of osteoclast count.

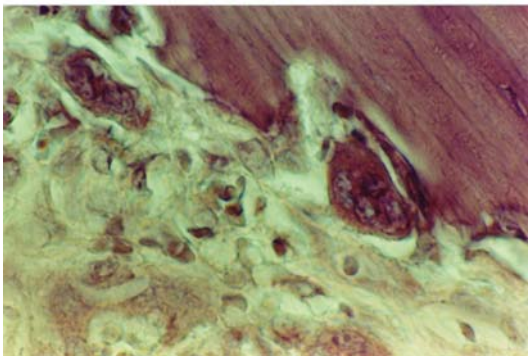

Fig. 4. Osteoclast under light microscope (×100).

Statistical analysis was carried out using SPSS 13 for Windows (LEAD Technologies, Inc, USA). One-sample Kolmogorov-Smirnov test was used to calculate the normal distribution of data and Levene's test was used to evaluate the homogeneity of variances. Finally, independent sample t-test was used to compare tooth movement and osteoclast counts in the samples.

Mauchly sphericity test was used to evaluate the co-variances of rats' weights at three different intervals. Repeated measures test was used to evaluate the significance of differences in rats' weights. Paired t-test was used to carry out multiple comparisons between rats' weights at three different intervals. Normally distributed variables have been reported as mean ± standard deviation.

Results

The means of rats' weights prior to the experiment were 191 ± 5 gr in the control group and 184 ± 5 gr in the experimental group. At spring placement time the means

On the 7th day after spring placement the means of tooth movement were 1.90 ± 0.34 mm in the control group and 1.58 ± 0.19 mm in the experimental group and the difference was statistically significant (p=0.025). On this day the number of osteoclasts in a 1-mm^2 surface area in the control group was 3.33 ± 0.66 and 2.6 ± 0.95 in the experimental group. The difference was at a significance level of $\alpha = 0.1$ (p=0.062).

of rats' weights were 204 ± 16 gr in the control group and 175 ± 8 gr in the experimental group. There was a significant difference between the weights of the rats due to psychological stress. ($P < 0.05$) (Fig. 5).

On the 14th day after spring placement the means of tooth movement were 2.36 ± 0.44 mm in the control group and 1.82 ± 0.34 mm in the experimental group and the difference was statistically significant (p<0.005). On this day the difference in the number of osteoclasts in a 1-mm^2 surface area between the two groups was not statistically significant (p=0.56) (Tables 1, 2).

Discussion

The evaluation of the influence of various factors on orthodontic tooth movement has appealed to a large number researchers. These factors have been studied and evaluated in an attempt to accelerate orthodontic tooth movement, to prevent root resorption and broadly to shorten the orthodontic treatment period and at the same time to minimize the risk of the complications resulting from the treatment.

Prior to discussion and conclusion it should be pointed out that to date no studies have been carried out into the role of psychological stress in the activity, proliferation and differentiation of osteoblasts, osteoclasts and proin-

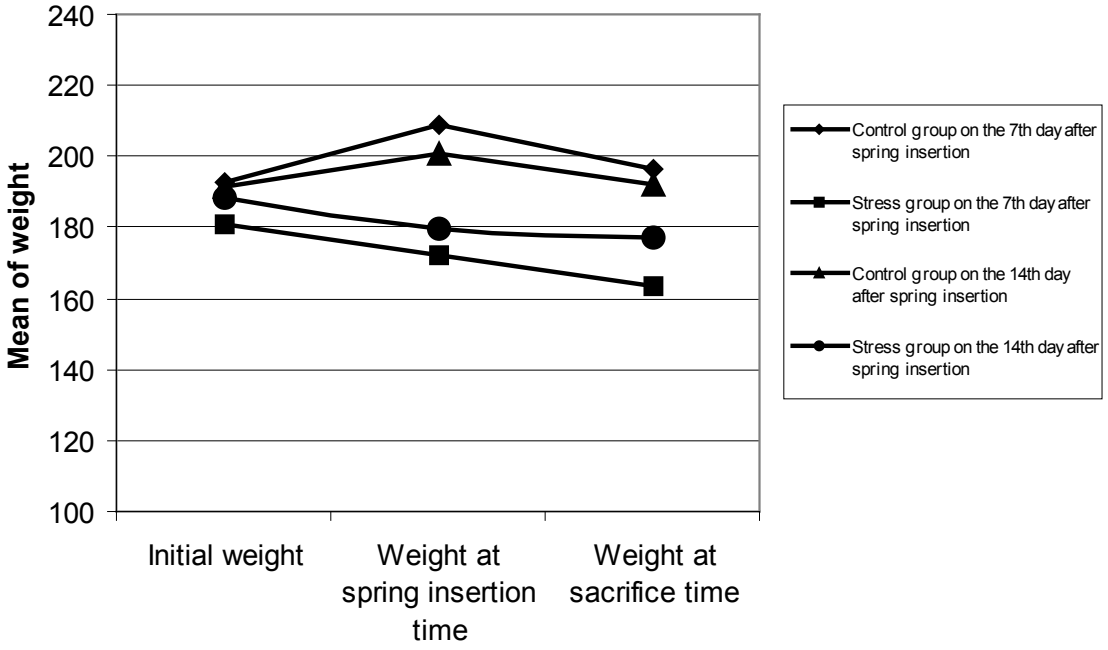

Fig. 5. Mean values of weights at three different intervals.

Table 1. Summary of tooth movement and osteoclast counts (1 mm^2).

	Initial weight	Weight at the spring insertion time	Weight at the sacrifice time
Control group (7th day after spring placement)	192.6 ± 16.5	209.1 ± 16.3	196.2 ± 15.8
Control group (14th day after spring placement)	191 ± 13.5	200.9 ± 16.5	191.9 ± 12.8
Experimental group (7th day after spring placement)	180.8 ± 11.6	172.3 ± 8.5	163.3 ± 5.3
Experimental group (14th day after spring placement)	188.2 ± 8.2	179.5 ± 8.7	177.3 ± 12.3

Values are means ± standard deviation. $p>0.05$ is not statistically significant.

Table 2. Summary of weight at three different intervals.

	Initial weight	Weight at the spring insertion time	Weight at the sacrifice time
Control group (7th day after spring placement)	192.6 ± 16.5	209.1 ± 16.3	196.2 ± 15.8
Control group (14th day after spring placement)	191 ± 13.5	200.9 ± 16.5	191.9 ± 12.8
Experimental group (7th day after spring placement)	180.8 ± 11.6	172.3 ± 8.5	163.3 ± 5.3
Experimental group (14th day after spring placement)	188.2 ± 8.2	179.5 ± 8.7	177.3 ± 12.3

Values are means ± standard deviation. $p>0.05$ is not statistically significant.

flammatory mediators at the site of tooth movement and the current study is a starter to extensive studies in this field. One of the purposes of this study was to (indirectly) elucidate the vague points in this path and to determine appropriate paths for future studies so that a number of serial studies could be carried out to solve the puzzle.

The results indicated that in the stress group the rats experienced weight loss ($p<0.05$). The results of this study coincide with the results of a study carried out by Spangenberg et al, who reported that psychological stress resulted in 14% weight loss in the stress group rats compared to the control group rats in 4 weeks (32). In a study the weight of the rats in the stress group was less than that in the control group (33). Other researchers have reported that psychological stress gives rise to weight loss in rats (34).

In the present study, psychological stress resulted in a decrease in osteoclastic count in the surface area under a light microscope on the 7th day after spring placement compared to the control group (at a significance level of $\alpha=0.1$).

PDL osteoclasts mainly originate from osteoclast progenitors in the bone marrow (35). Blood cortisol level increases in response to psychological stress. This hormone influences the immune system, reducing T lymphocyte and monocyte count (10,13). With a decrease in monocytic counts, considered osteoclast progenitors, a decrease in the number of osteoclasts in the surface area under a light microscope is expected in the rats in the stress group. In the present study, this depletion is reflected by a decrease in the distance between the mesial line angles of the central incisors of the rats in the psychological stress group. Researchers have demonstrated that psychological stress results in a decrease in IL-1 and IL-8 levels in the wound site (13,16). Other researchers reported that psychological stress gives rise to IL-6 and IL-1 level decrease in the wound site, leading to delayed wound healing (17). It is probable that these consequences can be found in the inflammation site, resulting from orthodontic tooth movement but further research is required to substantiate this. There is a possibility that principal chemical mediators involved in the orthodontic movement of teeth are affected by psychological stress, influencing orthodontic tooth movement. Further research into these proinflammatory mediators in tooth movement site can help solve these puzzles.

In the present study on the 14th day after spring placement, orthodontic tooth movement in the study group was less than that in the control group but the difference in the number of osteoclasts between the two groups on the same day was not statistically significant. This indicated that psychological stress reduces the number of osteoclasts at the site of tooth movement and this decrease in not linear, since on the 7th day after spring placement the number of osteoclasts decreased in the experimental group but this decrease did not continue from the 7th day to the 14th day. On the other hand, the difference in the amount of tooth movement between the two groups on the 14th day may be attributed to the influence of psychological stress on the principal proinflammatory mediators involved in orthodontic tooth movement.

As it was explained previously, orthodontic force involves both neural and immune systems and orthodontic tooth movement is an inflammation-like process (6). Psychological stress compromises the immune system (13-15); therefore, a decrease in the amount of orthodontic tooth movement in the experimental group in the present study can be explained by the immune system compromise and the subsequent disruption of inflammatory processes involved in tooth movement.

Conclusion

Psychological stress due to crowded environment-induced stress (housing density) and cat odour led to the following outcomes in the orthodontic movement of tooth in rats:

1) The amount of orthodontic movement of teeth in rats decreased due to psychological stress.

2) The number of osteoclasts around the root in the movement direction decreased due to psychological stress, but this process was not parallel with time and demonstrated a nonlinear decrease in the number of osteoclasts.

3) The rats experienced weight loss as a result of psychological stress.

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