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Original article

Evaluation of the effectiveness of intermittent mechanical pressure with short loading duration: new type of intermittent force for orthodontic treatment

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Summary

Background: Since it is difficult to precisely control the magnitude of force in orthodontic practice, controlling the duration of force is expected to prevent tissue damage. The use of an actuator as an appliance for intermittent force application is a possible solution for controlling the duration. However, effectiveness of an actuator for controlling the duration is still not clear.
Aim: To determine whether a short loading duration of intermittent force has the potential to maintain a sound condition of the capillaries and induce dilation of capillaries, which is a requisite phenomenon for orthodontic tooth movement.
Materials and methods: Six-week-old male hamsters with a dorsal skinfold chamber attached to an intermittent loading device were used. In three experimental groups, application of intermittent pressure that involved repetition of loading and unloading was performed. The durations of loading/unloading were 1 second/9 seconds (group T10), 1 second/19 seconds (group T20), and 1 second/29 seconds (group T30). Vessels were examined using a fluorescence microscope and a stereomicroscope for 5 days.
Results: Widths of capillaries in groups T10 and T20 increased significantly ($P < 0.01$). In contrast, widths of capillaries in group T30 showed no significant difference from those in the control group. Only group T10 showed bleeding, obvious destruction of vessels, and a significant increase in the rate of disappearance of vessels.
Conclusion: In the epidermis, although conditions are limited, a short loading duration of intermittent force maintains a sound condition of the capillaries at the tissue level and induces dilation of capillaries.

Introduction

In orthodontics, there has been much discussion about the relationship between tooth movement and periodontal tissue (1–8). Most of the discussion has been focused on the magnitude of force applied to teeth. However, in clinical practice, it is difficult to precisely control the magnitude of force (9), and the diversity in anatomical shapes of the roots of teeth and alveolar bone makes control of the magnitude of force even more complicated. Therefore, heavy force is often unintentionally applied, and root resorption as well as undermining bone resorption is likely to occur in such areas (9).

It is known that monocyte–macrophage lineage cells emerge from post-capillary venules to the bone surface (10) and differentiate into osteoclasts (11), which are essential (12) for orthodontic tooth movement. Recent studies have revealed that mechanical stress induces dilation of a capillary to the size of a post-capillary venule and that leukocyte recruitment occurs in these dilated capillaries (13–15). This phenomenon is requisite for the orthodontic tooth movement. Thus, an optimum orthodontic force requires the features to induce dilation of capillaries while maintaining a sound condition of the tissues even if heavy force is applied unintentionally. As an optimum orthodontic force, we focused on intermittent force to avoid a long period of ischemia. However, it is difficult to control the loading duration. Therefore, we invented an automatic intermittent loading device that is able to apply mechanical pressure for several seconds. The aim of this study was to determine whether a short loading duration of intermittent force has the potential to maintain a sound condition of the capillaries and to induce dilation of capillaries.

Materials and methods

Animals

Twenty-six-week-old male Syrian golden hamsters (mean body weight of approximately 120 g) were used as experimental animals. Five hamsters without mechanical loading were used as controls. In three experimental groups (five hamsters in each group), application of intermittent pressure that involved repetition of loading and unloading was performed. The durations of loading/unloading for the three groups were 1 second/9 seconds (group T10), 1 second/19 seconds (group T20), and 1 second/29 seconds (group T30). During the experiment, the hamsters were fed in plastic cages with bedding chips and had free access to water and pellets. The experiment was approved by the Animal Care and Use Committee of Hokkaido University (14-0044) and was conducted with strict adherence to the Guidelines for Animal Experiments of Hokkaido University.

Methods

Catheterization

Under anaesthesia with diethyl ether (Kanto Chemical Co., Inc., Tokyo, Japan) and thiamylal sodium (2.5 per cent isozol; Mitsubishi Pharma Corp., Osaka, Japan) intraperitoneally injected at 2.5 ml/kg body weight, an incision was made in the right side of the neck and a catheter (diameter: 0.5 mm; KN-392 SP8; Natsume Seisakusho Co., Ltd, Tokyo, Japan) was inserted into the jugular vein. The catheter was fixed and passed through the back of the hamster to the outside of its body (16). Gentamicin (Gentacin Injection 40, MSD Co., Inc., Tokyo, Japan) at 2 mg/kg body weight was administered through the catheter, and buprenorphine (Lepetan injection 0.3 mg; Otsuka Pharmaceutical Co., Ltd, Tokyo, Japan) at 0.01 mg/kg body weight was administered by intramuscular injection.

Implantation of a dorsal skinfold chamber with a loading device

The dorsal skinfold chamber consisted of two frames of acrylic plate. One plate was prepared for an observation window and the other was prepared for a loading device (Figure 1).

Under anaesthesia, the back of the hamster was shaven carefully and the hamster was placed in the prone position. The shaved skin was extended and sandwiched at the midline. The acrylic plate with the loading device was fixed to the right side of the hamster. After the hamster had been placed in the lateral position, skin layers on the left side were completely removed to the size of the observation window (diameter: 15 mm) and the acrylic plate with the observation window was fixed on the left side using connecting screws. The distance between the two acrylic plates (pinched skin width) was fixed at 2 mm. The inner tissue layers on the right side could be seen through the window (17). At the moment of implantation of the chamber, blood flow of the microvasculature was checked using a microscope. Implantation of a chamber with the loading device was also performed in the control group.

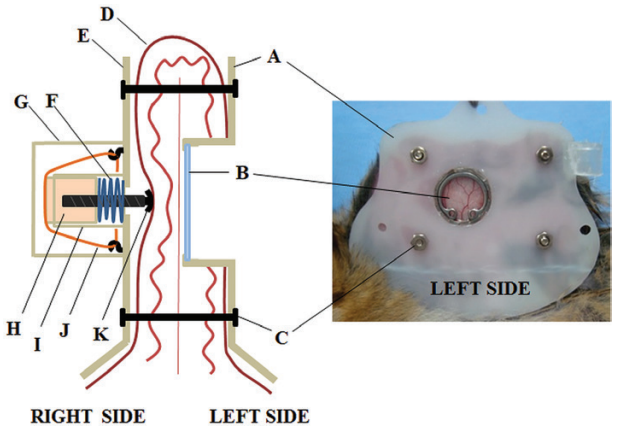

Figure 1. Diagram of the dorsal skinfold chamber with the loading device. The inner tissue layers on the right side could be seen through the window. A: acrylic plate with observation window; B: cover glass (diameter: 12 mm); C: connecting screw; D: dorsal skin; E: acrylic plate with the loading device; F: spring (Ni-Ti); G: protection cover of the loading device; H: inner cylinder of the loading device; I: outer cylinder of the loading device; J: actuator (Biometal Fiber); K: apex of the loading device.

A shape memory alloy actuator (Biometal Fiber; Toki Corp., Tokyo, Japan) that contracts by the passage of electricity was used as the loading device. Contraction of the actuator caused vertical motion (movement range: 0.5 mm) of a lingual button (round shape of 2 mm in diameter, Micro-Loc lingual button; Tomy Inc., Tokyo, Japan) against the acrylic plate. The strength of this motion was adjusted to 9 gf at top of the device by using a Ni-Ti coil spring (Tokaibane MFG. Co., Ltd, Tokyo, Japan). The loading/unloading duration was regulated precisely by an electronic circuit using a timer IC (NE555P; Texas Instruments Inc., Dallas, Texas, USA).

Power supply and an electronic circuit were set on the cage and connected to the loading device with copper wires. The hamster was fed in the cage under a free condition by using a slip ring device (PLR27; Nidec Copal Corp., Tokyo, Japan).

Observations and recordings

Observations and recordings were performed after 48 hours of rest from surgery, so that anaesthesia and surgery would not affect microcirculation (17). After the first day of observations and recordings (day 0), application of intermittent mechanical pressure to the epidermis was started. Observations and recordings were performed every 24 hours until day 5. During observations and recordings, the hamster was fixed on the stage of a microscope using a fixing device, and all procedures were performed without anaesthesia. Stereomicroscopic observation and recordings were performed using a stereomicroscope (SZ61; Olympus Corp., Tokyo, Japan) with a CCD camera (DP22; Olympus Corp., Tokyo, Japan). Fluorescence microscopic observation and recordings were performed using a fluorescence microscope (BX50; Olympus Corp., Tokyo, Japan) with

a fluorescence cube (U-NIVA2; Olympus Corp., Tokyo, Japan) and CCD camera (CS230; Olympus Corp., Tokyo, Japan) after injection of fluorescein isothiocyanate–dextran (FITC–dextran; M.W. 150 000; Sigma Chemical, St Louis, Missouri, USA) at 100 mg/kg body weight through the catheter within 15 minutes. The same region as that on day 0 was used for observations on days 1–5, and images were stored on a computer (Surface; Microsoft, Redmond, Washington, USA). During the experiment, the control group was treated in the same way as the experimental groups except for application of intermittent mechanical pressure.

Evaluations

In this study, vessels smaller than 8 μm in diameter were classified as capillaries, and vessels of 8 to 30 μm in diameter were classified as post-capillary venules (18). The term 'microvasculature' is used as vasculature composed of small vessels.

Dynamics of vessels

Stereomicroscopic images were used for examining dynamics of the microvasculature, and fluorescent microscopic images were used for examining dynamics of the capillaries. Dilation of vessels, condition of vessel structure, and other responses were examined qualitatively.

Changes in widths of capillaries

A measurement tool of image processing software (Adobe Photoshop CC 2014; Adobe Systems Inc., San Jose, California, USA) was used for measurement of the widths of capillaries (19) (Figure 2). From FITC–dextran images, the same capillary with blood flow on days 0–5 was selected randomly (six capillaries from each hamster). In each capillary, measurement was performed at 10 randomly selected spots. The average value of 10 measurement results was used as the width of the capillary (15). The rate of change was calculated by setting its width on day 0 as the basis. Data for each hamster were processed, and significant differences were detected by ANOVA and the Tukey-HSD test (P value < 0.01) after confirming normality by the Shapiro–Wilk test.

Disappearance rate of capillaries

FITC–dextran images obtained on day 0 and day 5 were used for evaluating the disappearance of capillaries (Figure 2). Two vessel bifurcations were selected on the image of day 0. These bifurcations were identified on the image of day 5 as well. We observed the capillaries between these two vessel bifurcations. By comparing the images of day 0 and day 5, the number of capillaries that had disappeared was counted. The rate of disappearance was calculated by dividing the number of capillaries that had disappeared by the number of capillaries on day 0. Five pairs of images were used for each hamster. Significant differences were detected by ANOVA and the Tukey-HSD test (P value < 0.01) after confirming normality by the Shapiro–Wilk test.

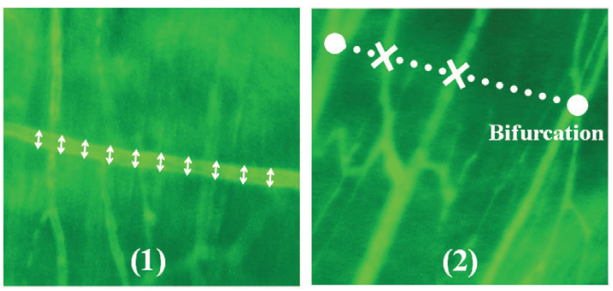

Figure 2. Evaluation method. (1) Width of a capillary: In a capillary, measurement was performed at 10 randomly selected spots. The average value of 10 measurement results was used as the width of the capillary. (2) Disappearance of capillaries: Two vessel bifurcations were selected randomly. Capillaries between these bifurcations were observed (X mark). By comparison with the image obtained on day 0, the number of capillaries that had disappeared in the image obtained on day 5 was counted.

Results

Dynamics of the microvasculature

Control group

The observation area became reddish (Figure 3). However, there was no major change in the microvasculature.

Group T10

Under the loading region, the microvasculature had disappeared and the area became ischemic (arrow). In the surrounding region, the microvasculature was dilated and tortuousness was emphasized (arrowhead). However, the dilated microvasculature started to shrink after the peak of dilation (days 2–3). On day 5, most of the microvasculature was difficult to observe. As shown in Figure 5, there was one case with bleeding under the loading region (arrow in Figure 5).

Group T20

Blood flow was maintained during the whole observation period (arrow). No ischemic condition or bleeding was observed. Some of the microvasculature became clearly observable (arrowhead).

Group T30

Blood flow was maintained during the whole observation period (arrow). No ischemic condition or bleeding was observed.

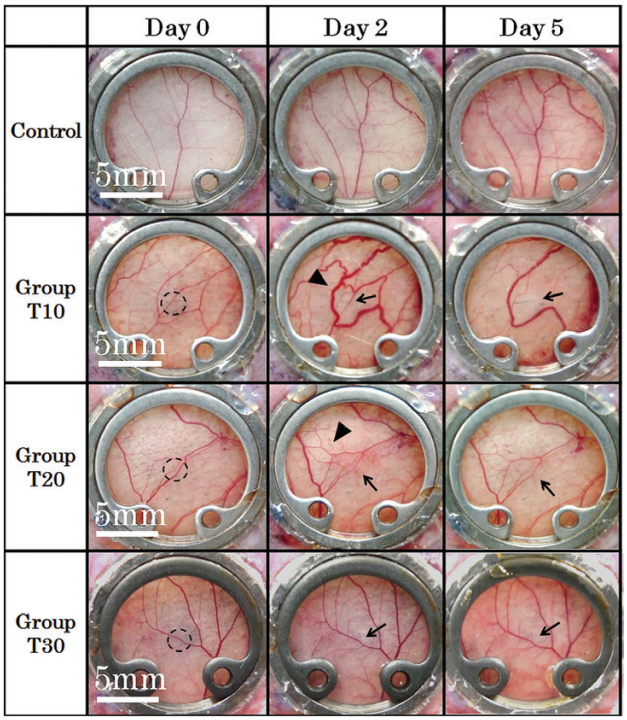

Figure 3. Dynamics of the microvasculature (broken circle: loading region). Group T10: An ischemic condition was observed only in group T10 (arrow). However, some of the capillaries dilated remarkably (arrowhead). Groups T20 and T30: Blood flow was maintained in both groups (arrow).

Dynamics of the capillaries

Control group

There was no major change in the size or orientation of capillaries (Figure 4).

Group T10

Most of the capillaries became difficult to observe (arrows). However, some of the capillaries showed remarkable dilation (arrowhead). As shown in Figure 5, some of the vessels dilated remarkably on days 1–2, and leakage of FITC–dextran occurred due to loss of vessel structure (arrowhead in Figure 5).

Group T20

Most of the capillaries observed on day 0 gradually dilated (arrows).

Group T30

As in the control group, there was no major change in the size or orientation of capillaries throughout the experimental period.

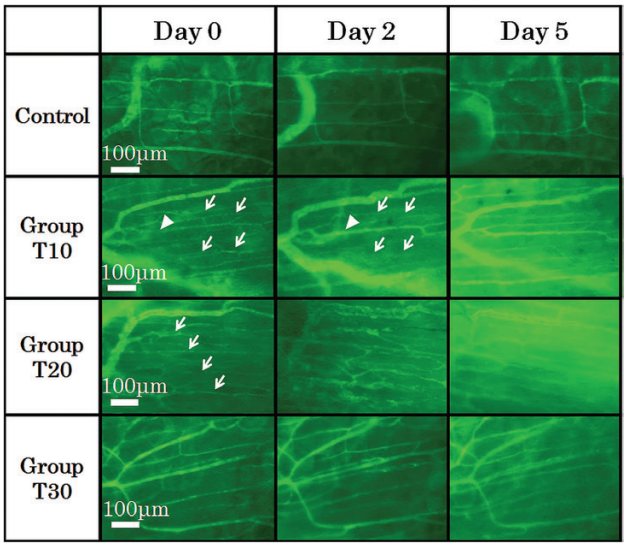

Figure 4. Dynamics of capillaries. Group T10: Most of the capillaries became difficult to detect (arrows). However, some of the capillaries dilated remarkably (arrowhead). Group T20: Most of the capillaries dilated gradually (arrows). Group T30: There was no major change in the size or orientation of capillaries.

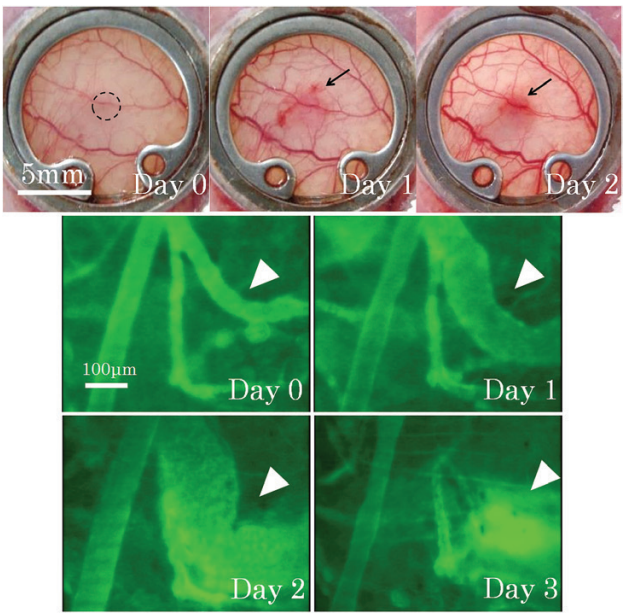

Figure 5. Destruction of vessel structure in group T10. Stereomicroscopic observation: Bleeding was observed under the loading region only in group T10. Fluorescent microscopic observation: Some of the vessels dilated remarkably on days 1–2. On day 3, leakage of fluorescein isothiocyanate (FITC)–dextran occurred due to loss of vessel structure.

Changes in widths of capillaries

The mean width of capillaries observed on day 0 was 6.1 ± 0.5 μm (Figure 6).

The control group showed a slight increase and its peak (day 4) was +13 per cent. Groups T10 and T20 showed similar changes. Widths of capillaries in groups T10 and T20 increased on days 1–3 and decreased after their peaks on day 3. Rates of changes at their peaks were +74 per cent in group T10 and +55 per cent in group T20. The rate of change in group T10 was significantly higher ($P < 0.01$) than other groups on days 1–3. However, on days 4–5, there was no significant difference between groups T10 and T20. The rate of change in group T20 was significantly higher ($P < 0.01$) than in the control group and group T30 on days 2–5. The rate of change in

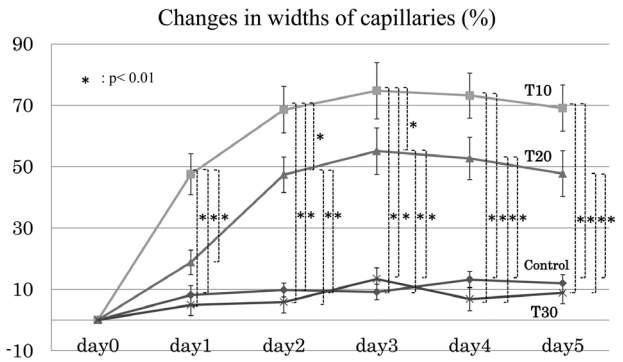

Figure 6. Changes in widths of capillaries compared to day 0. Control group and group T30 showed a slight increase. Groups T10 and T20 showed remarkable increases, but there was no significant difference between groups T10 and T20 on the last 2 days.

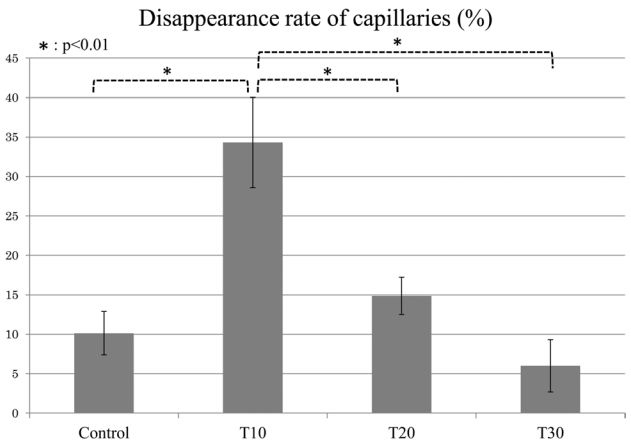

Figure 7. Disappearance rate of capillaries. The rate of disappearance of capillaries in group T10 was significantly higher than the rates in other groups.

group T30 was similar to that in the control group. Therefore, on days 1–5, there was no significant difference between group T30 and the control group.

Disappearance rates of capillaries

In the control group, 10 per cent of the capillaries had disappeared on day 5 compared to day 0 (Figure 7). The rate of disappearance in group T10 was 34 per cent, which was significantly higher ($P < 0.01$) than the rates in other groups. The rate of disappearance in group T20 was 14 per cent, which was not significantly different from the rates in the control group and group T30. The rate of disappearance in group T30 was 5 per cent, which was lower than that in the control group but not significantly different.

Discussion

Conventional strategy for preventing tissue damage in the current orthodontic practice is to control the magnitude of force rather than to control the duration of force. In controlling the magnitude of force, we think that the most important point is to measure the magnitude of force applied to the periodontal ligament. However, that is the most difficult point in the clinical practice due to the diversity in anatomical shapes of the teeth and alveolar bone (20). On the other hand, advancement in technology enables us to control the duration of force. Therefore, to maintain a sound condition of the tissue, it will be more reasonable to control the duration than the magnitude.

It is difficult to observe chronological changes of the same periodontal ligament. This is because the periodontal ligament is located between hard tissues, cementum and alveolar bone. In the dorsal skin chamber technique, the epidermis is sandwiched between two hard plates just like a periodontal ligament. This enables observation of chronological changes of the same tissues. Therefore, we used a dorsal skinfold chamber as a model for the periodontal ligament. A recent study has revealed that mechanical stress induces dilation of a capillary. Leukocyte recruitment occurs when the capillary has dilated to the size of a post-capillary venule (13–15). Since this phenomenon is requisite for orthodontic tooth movement, diameter changes were used as criteria for assessment of orthodontic tooth movement conditions in this study. It is known that tissue damage increases in proportion to loading duration (21), and we chose 1 second as the loading duration in order not to cause ischemia. A longer duration of unloading is thought to provide adequate time for recovery from compression and prevent ischemia. A recent study using finite element analysis revealed that 32 kPa (326 gf/cm^2) of stress is applied to the root apex at the moment of orthodontic tooth movement (22). Another study using finite element analysis revealed that 2.82–4.24 N/cm^2 (287–432 gf/cm^2) of the stress is applied, and root resorption is thought to occur at the stress-concentrated site (23). Therefore, a force magnitude of 9 gf (300 gf/cm^2) at the top of the device (round shape, 2 mm in diameter) was used in this study.

In both groups T10 and T20, widths of capillaries increased to post-capillary venules. These dilated capillaries are expected to become sites for recruitment of leukocytes (13–15). There were significant differences between groups T10 and T20 on days 1–3 but not after day 4, indicating that there is no significant difference in the viewpoint of vessel width after several days of loading. However, from viewpoints of damage caused in tissue, it is obvious that group T10 had a greatly damaged microvasculature, which became difficult to observe. With the magnitude of force used and the high loading frequency, the duration of unloading in group T10 is thought to be insufficient for recovery from damage under the loading region, even though the loading duration was only 1 second. Longer reperfusion in group T20 after loading is thought to be an appropriate duration for recovery from damage, and this period of reperfusion is thought to prevent severe damage of the capillaries in a tissue level. T30 did not cause severe damage of the capillaries in a tissue level, but morphological change of the capillaries was not observed.

Limitations

Several limitations have to be considered in this study.

1. In order to observe chronological changes of the same region, we used a dorsal skinfold chamber as a model of the periodontal ligament. Although the components of the periodontal ligament and dorsal skin are similar, there might be some differences. Therefore, further study on experimental tooth movement is needed to clarify the effect of a short loading duration of intermittent pressure.
2. Evaluation of tissue damage was performed only by capillary assay at the tissue level in this study.
3. For clinical application of the actuator used in this study, there are several problems (e.g. power supply, insulation) to be solved for oral application. Although there are several problems to be solved, the coil spring made of actuator used in this study already exists, and it is possible to work as elastic chain.

Conclusion

In the epidermis, although conditions are limited,

1. A short loading duration of intermittent force maintains a sound condition of the capillaries at a tissue level.
2. A short loading duration of intermittent force induces dilation of capillaries, which is a requisite phenomenon for orthodontic tooth movement.

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Conflict of interest

The authors declare no conflict of interest or financial relationship with any organization or material used in the study.

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