Effect of minocycline on induced glial activation by experimental tooth movement

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Introduction: Orthodontic tooth movement causes pain to a patient. Glial cells are nonneuronal cells in the central nervous system and are implicated in various types of pain. In this study, we assessed glial activation responses after experimental tooth movement using immunocytochemical detection of anti-CD11b (OX42) and glial fibrillary acidic protein immunoreactivity to illustrate the microglial and astrocytes response, respectively. In addition, the effect of minocycline in reducing pain during tooth movement was also investigated. **Methods:** Fifty-five Sprague Dawley rats with and without administration of minocycline after 1, 3, 5, 7, and 14 days (n = 5, for each) of tooth movement were used. Immunohistochemistry for microglia (OX42) and astrocyte (glial fibrillary acidic protein) were performed at the medullary dorsal horn (trigeminal subnucleus caudalis). Three-dimensional quantitative analysis was performed with a confocal fluorescence microscope and a software program. **Results:** There was a significant increase in the OX42 and glial fibrillary acidic protein immunoreactivity in response to tooth movement in the medullary dorsal horn. Furthermore, systematic administration of minocycline, a selective inhibitor of microglial activation, significantly attenuated the nociceptive c-Fos expression in the medullary dorsal horn that was induced by experimental tooth movement. **Conclusions:** These data indicate the possible importance of microglial activation in the development of orthodontic pain. This is also the first report on the systematic application of minocycline. (Am J Orthod Dentofacial Orthop 2016;149:881-8)

Orthodontic force causes continuous pressure on the teeth and supporting structures that induces pain.[1] Significant pain tends to appear normally about half a day later and then lasts for a few days, indicating that orthodontic pain is different from immediate acute pain.[2-4] Orthodontic patients feel discomfort,[5,6] and 95% of them experience varying degrees of discomfort during treatment.[7]

Glial cells are nonneuronal cells in the central nervous system (CNS) that have a role in the initiation and maintenance of persistent pain states.[8] Microglia and astrocytes have been implicated in various types of pain such as subcutaneous inflammation and peripheral nerve injury.[9] Microglia are the early responding glial cells in the CNS after injury, and microglia release products that activate astroglia.[10,11] Furthermore, both microglia and astrocytes have the ability to release proinflammatory cytokines.[12,13] These proinflammatory cytokines and prostaglandins released after microglial activation play a role in central sensitization and are implicated in exaggerated pain states.[14-16]

Because orthodontic pain is initiated by an inflammatory-like response in the periodontal ligament, nonsteroidal anti-inflammatory drugs are the preferred over-the-counter medications for orthodontic pain.[17,18] The major concern regarding these drugs is the interference produced on inflammation associated with tooth movement.[19] Since nonsteroidal anti-inflammatory drugs cannot cross the blood-brain barrier, they directly react with the peripheral tissues. The current

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trend is directed toward use of preemptive or preoperative analgesics, which are administrated at least 1 hour before every orthodontic procedure.[20] Preemptive analgesia blocks the afferent nerve impulses before they reach the CNS, abolishing the process of central sensitization. There are only a few suggested preemptive drugs, most of which inhibit bone resorption and interfere with tooth movement.[20,21] Orthodontic pain characteristically is long lasting but is not persistently acute. Thus, preemptive analgesia targeting the brain or brainstem might relieve the orthodontic pain efficiently. It may be productive to develop a new preemptive analgesia that blocks neuronal hyperactivity input to the CNS that would reduce pain or discomfort without tooth movement side effects.

Minocycline is a semisynthetic tetracycline derivative that exerts anti-inflammatory effects completely distinct from its antimicrobial action.[22] Minocycline is a lipophilic molecule and readily crosses the blood-brain barrier[23] and selectively disrupts the activation of microglia without directly affecting neurons or astroglia.[15,24] Minocycline's antihyperalgesic and antiallodynic effects have been demonstrated in models of arthritis, spinal nerve transection, and sciatic inflammatory neuritis.[15,25,26] Interestingly, minocycline has no effect on acute inflammation and attenuates and delays the development of neuropathic pain[27] and formalin-induced inflammatory pain responses.[28] Lasting nociception induced by experimental tooth movement may lead to glial activation at the medulla, and glial inactivation by minocycline medication should show antineuronal activity.

In this study, anti-CD11b (OX42) and glial fibrillary acidic protein (GFAP) were used as glial cell markers and for microglial and astrocytic responses, respectively. Proto-oncogene c-Fos, known as a marker for nociceptive pain, was also used for double immunostaining with glia markers.

Our hypothesis was that there would be no significant difference in the OX42 immunoreactivity, GFAP immunoreactivity, and c-Fos immunoreactivity with and without the application of minocycline after experimental tooth movement in rats.

MATERIAL AND METHODS

The animal committee of the Graduate School of Medicine and Dentistry at Okayama University in Japan approved the experimental protocol.

Fifty-five adult Sprague Dawley rats (weight, 150-200 g; CLEA Japan Inc., Tokyo, Japan) (5 control; 25 with minocycline; 25 without minocycline) were used for the study. For the tooth movement, the rats were anesthetized with a mixture of pentobarbital sodium (20 mg/kg) and ethylcarbamate (650 mg/kg), and a piece of an orthodontic elastic module was inserted between the maxillary first and second molars on the right side (Fig 1, *A*).[29] After 1, 3, 5, 7, and 14 days (each group, n = 10) after tooth movement, the rats were anesthetized by inhalation to a depth at which respiration was markedly suppressed and then transvascularly perfused with 50 mL of saline solution followed by 500 mL of 4% formaldehyde in 0.1 mol per liter of sodium phosphate buffer (pH, 7.4). The lower brain stem was dissected out, immersed in the same fresh fixative overnight, and then cryoprotected by immersion in 20% sucrose in 0.02 mol per liter of phosphate-buffered saline solution at 4°C. The frozen sections were embedded in an optimal cutting temperature compound and cut frontally (50 μm thick).

In 25 rats, minocycline was administrated during tooth movement. Minocycline (Sigma-Aldrich, St Louis, Mo) was dissolved in sterile water and sonicated to ensure complete solubilization. The rats received an intraperitoneal injection of vehicle or minocycline (50 mg/kg) once a day during the experimental period. They were transvascularly perfused followed by dissection 1, 3, 5, 7, and 14 days after tooth movement.

For the immunohistochemistry, microglia were identified by OX42 (mouse anti-rabbit, 1:1000; Sigma-Aldrich), which recognizes cell surface complement receptor 3.[30] Astrocytes were identified by anti-GFAP (mouse monoclonal, 1:5000; Dako, Glostrup, Denmark), which is an intermediate filament protein predominantly expressed in cells of astroglial origin. The synthesis of this protein is activated by injury or stimuli.[31] c-Fos immunoreactivity was also analyzed to evaluate the orthodontic nociception at the medulla (1:10,000; Oncogene Science, Cambridge, Mass).[32] The avidin-biotin-horseradish peroxidase complex method was used, and all sections were incubated with 1% bovine serum albumin (Amersham International, Amersham, United Kingdom) and 0.3% Triton-X 100 (Sigma-Aldrich) in phosphate-buffered saline solution for 1 hour at room temperature. Sections were then incubated overnight at 4°C with an OX42, GFAP, or c-Fos antibody. The sections were developed using a mixture of Alexa Fluor 594-conjugated goat anti-rabbit IgG (Invitrogen, Carlsbad, Calif) and Alexa 488-conjugated goat anti-mouse IgG (Invitrogen). Some sections were exposed to avidin-biotin-horseradish peroxidase complex in 0.1 mol per liter of PBST for c-Fos immunostaining for quantitative analysis. The antigens were visualized by reaction with 0.05% 3,3-diaminobenzidine tetrahydrochloride in phosphate-buffered saline solution and 0.003% hydrogen peroxide for 5 minutes. The