Original Article

Effect of Celecoxib on Emotional Stress and Pain-Related Behaviors Evoked by Experimental Tooth Movement in the Rat

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ABSTRACT

Objective: To test the efficacy of an animal model of pain and stress and evaluate the effects of celecoxib administered when orthodontic force is applied.

Materials and Methods: A 20-g reciprocal force was applied via an orthodontic appliance to the maxillary left first and second molars of 7-week-old male Sprague-Dawley rats. Rat behavior was evaluated at 5, 24, and 48 hours after the appliance was set. Behavior was assessed in a test field by the number of lines crossed in the first 30 seconds and 5 minutes following force application; number of lines crossed to the center; rearing time; and facial grooming time. Experimental group 1 received intraperitoneal administration of 30 mg/kg celecoxib before every behavioral test. Experimental group 2 received 90 mg/kg before the first behavioral test, and physiologic saline was administered before the remaining behavioral tests. Control groups received saline before every behavioral test and were given passive (passive control group) and active (active control group) appliances, respectively.

Results: Parameters related to pain increased in the active controls, whereas the parameters in the experimental groups decreased to the level seen in the passive controls. Statistically significant differences in pain-related behavior between control and experimental groups were found at 5 and 24 hours after placing the appliance. Stress-related behavior was significantly less in the experimental groups compared to the active control group during experimental periods.

Conclusions: The administration of celecoxib relieves pain- and stress-related behavior evoked by orthodontic tooth movement in the rat. This model might be a useful tool for the evaluation of pain and stress. (*Angle Orthod.* 2009;79:1169–1174.)

KEY WORDS: Pain; Celecoxib; Tooth movement; Behavior

INTRODUCTION

Pain and discomfort are frequent undesirable side effects of orthodontic treatment. Pain, one of the cardinal signs of inflammation, is almost inevitable and, for the patient, the most unpleasant reaction to orthodontic therapy. It begins a few hours after the application of an orthodontic force and lasts for approximately 5 days.[1–4] Some clinical reports show that most patients report pain and discomfort during the first few days of treatment.[4–6]

As far as we know, for orthodontics, the most effective way to reduce pain is through the use of analgesic agents. Selective cyclooxygenase-2 (COX-2) inhibitors were originally developed as chronic pain medications that offered the pain-relieving benefits of selective nonsteroidal anti-inflammatory drugs (NSAIDs) with fewer adverse gastrointestinal effects. Their demonstrated efficacy in treating postsurgical and acute medical pain has expanded their use. The theory behind the preoperative administration of selective COX-2 inhibitors is that inhibition of COX-2–mediated prostaglandin synthesis reduces nociceptive pain and prevents inflammatory-induced hyperalgesia.[7]

Celecoxib, a COX-2–selective NSAID, has been

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shown to be effective in relieving pain associated with orthopedic surgery,[8] arthroscopic knee surgery,[9] and ankle sprain,[10,11] as well as dental surgery.[9–11] Celecoxib has been shown to be well tolerated and associated with a lower incidence of endoscopically detected gastric and duodenal ulcers than nonselective NSAIDs such as aspirin and its derivatives. In addition, unlike nonselective NSAIDs, celecoxib does not interfere with normal platelet function or prolong bleeding time,[12] making celecoxib less likely to exacerbate bleeding during the treatment of acute pain following surgery or trauma.[13]

The use of preoperative analgesics provides blockage of afferent nerve impulses before they reach the central nervous system. When NSAIDs are given before any procedure, the body absorbs the NSAID before tissue damage occurs with subsequent prostaglandin production. NSAID application before oral surgery has been reported to decrease the pain intensity and delay the onset of pain and peak pain levels.[14] It has been shown that the administration of 400 mg of celecoxib before surgery is as effective as when it is administered postoperatively in major plastic surgery cases.[15]

The purpose of this study was to investigate the effect of celecoxib on pain and stress during tooth movement by observation of behavior in an animal model and to validate an animal model of pain.

MATERIALS AND METHODS

Twenty-eight male Sprague-Dawley rats (210–350 g) were housed in pairs in plastic cages in a colony room following a 12-hour light/dark cycle with the average room temperature maintained at 21°C to 23°C. Food and water were available ad libitum. After arrival, the rats were allowed to habituate to the experimental facilities for 1 week before the experiments began. The experimental procedures followed the Guidelines for Animal Research of the Animal Welfare Committee of Nagasaki University.

The animals were divided into four groups: two control groups and two experimental groups. The first drug administration took place 1 hour before setting the orthodontic appliance. Experimental group 1 was administered 30 mg/kg celecoxib intraperitoneally (i.p.) three times before every behavioral test. Experimental group 2 was administered 90 mg/kg celecoxib i.p. before the first behavioral test and was administered the same volume of saline i.p. before the remaining behavioral tests. The control groups received the same volume of saline i.p. before each behavioral test and had passive or active appliances set (passive and active controls) (Figure 1). The animals were weighed before treatment and at regular intervals during the experimental period.

The orthodontic appliance was set while the animals were under general anesthesia. The rats were injected i.p. with ketamine hydrochloride 87 mg/kg (Ketalar 50, Sankyo Co Ltd, Tokyo, Japan) in combination with xylazine hydrochloride 13 mg/kg (Celactal 2%, Bayer-Japan Co Ltd, Tokyo, Japan). The wire used for both active and passive appliances was a work-hardened titanium-nickel alloy measuring 0.228 mm in diameter and 14 mm in length (Tomy, Tokyo, Japan) (Figure 1). The appliance was set as previously described.[16] Briefly, buccopalatal grooves were cut with a steel bur (no. 0.5, Maillefer, Ballaigues, Switzerland) on the occlusal surfaces of the maxillary right first (M1) and second (M2) molars. The wire to be used for the passive appliance was bent at its center (by loop-forming pliers) and heat-treated so that the ends were 3 mm from one another, and each groove was just deep enough to seat the spring wire (0.3 mm). The site was dried, etched with 65% phosphoric acid for 20 seconds, rinsed with water, and dried. The wire used as an active spring (experimental group) was initially straight (not bent). An initial force of 20 g was delivered by the active appliance. In preparation for bonding the spring, the tips were brought together and maintained at a distance of 3 mm by a circular frame. The frame was removed to activate the spring after the spring was bonded. Finally, the springs were seated into the occlusal grooves and bonded into place with cyanoacrylate glue. The rats were then allowed to recover from anesthesia and returned to their cages in the colony room.

Behavioral Evaluation

Behavioral evaluation followed the same protocols as a previous study.[16] The tests were performed during the light phase of the light/dark cycle at 5, 24, and 48 hours after the appliances were placed. The rats were evaluated individually and returned to the colony room immediately afterward. The test room was quiet and temperature controlled (22°C). A video camera was positioned vertically 2 m above the test field. The test field consisted of 70- × 70-cm acrylic glass divided by white adhesive tape into 36 squares of identical size surrounded by 30-cm-high cardboard walls. The rat was placed at one corner of the open field. The following parameters were analyzed: (1) number of lines crossed during the first 30 seconds, (2) total number of lines crossed in 5 minutes, (3) number of lines crossed to the center of the open field, (4) rearing time, and (5) facial grooming time. A line was considered crossed when all four paws crossed it. Rearing time and facial grooming time were measured with a stop-

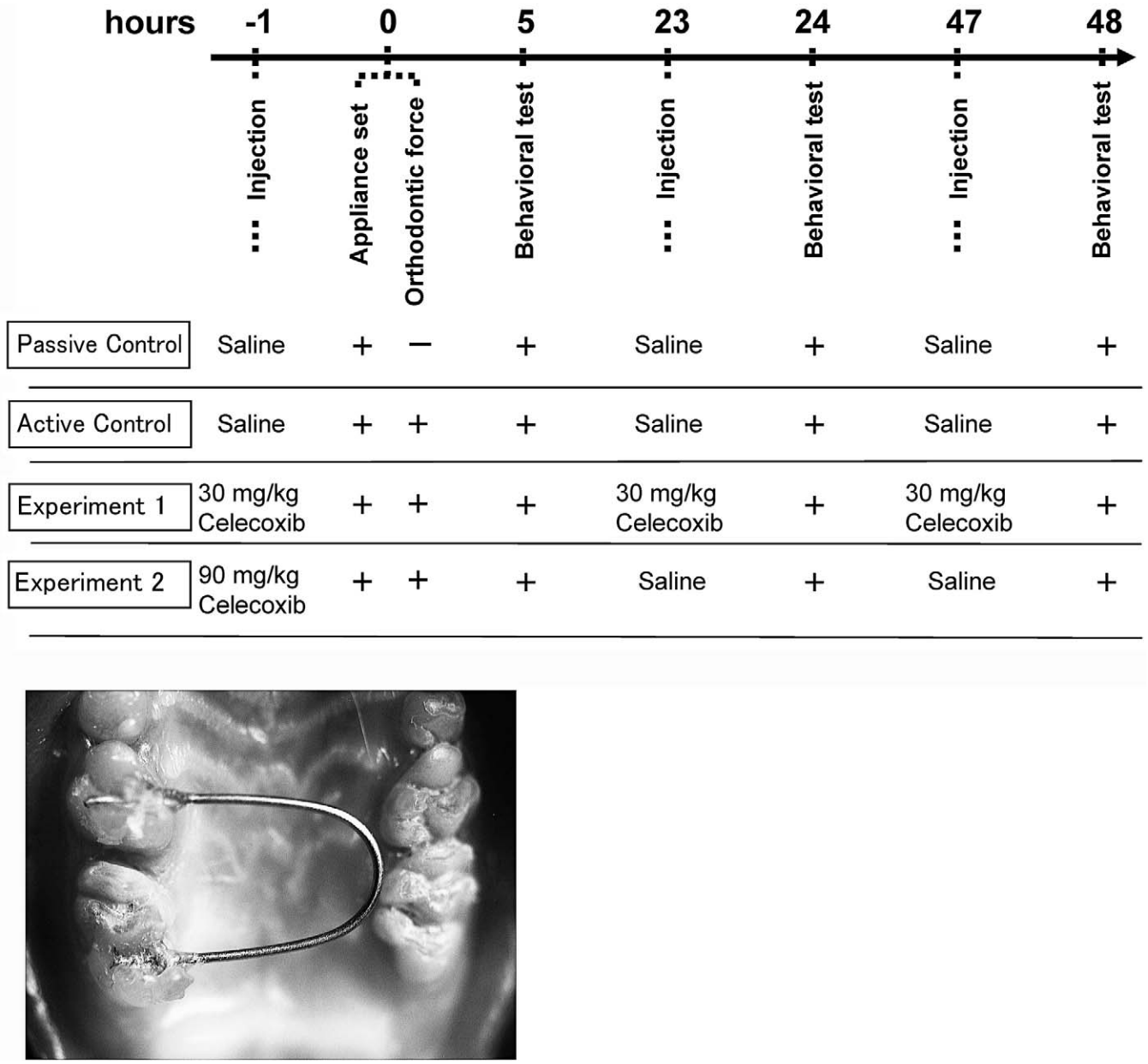

Figure 1. Study design (*above*) and appliance in situ (*below*). Passive control group, active control group, experimental group 1, and experimental group 2.

watch and consisted of the cumulative time of rearing and facial grooming episodes, respectively. In all tests, the animal's response was recorded on videotape and later analyzed by an observer blinded to the animal's group assignment.

After the open-field test, a resistance-to-capture test was performed. The test consisted of measuring the animals' resistance to being picked up by the examiner. The level of resistance was evaluated as follows: 0 indicates easy to pick up; 1, vocalizes or shies away from hand; 2, shies away from hand and vocalizes; 3, runs away from hand; 4, runs away and vocalizes; 5, bites or attempts to bite; and 6, launches a jump attack.

Data Analysis

Data from control and experimental groups were compared by the Scheffé test. The significance level was set at $P < .05$. Data are displayed as mean values $\pm$ standard error of the mean.

RESULTS

The initial and final body weights did not differ significantly in any groups. In the passive control group,

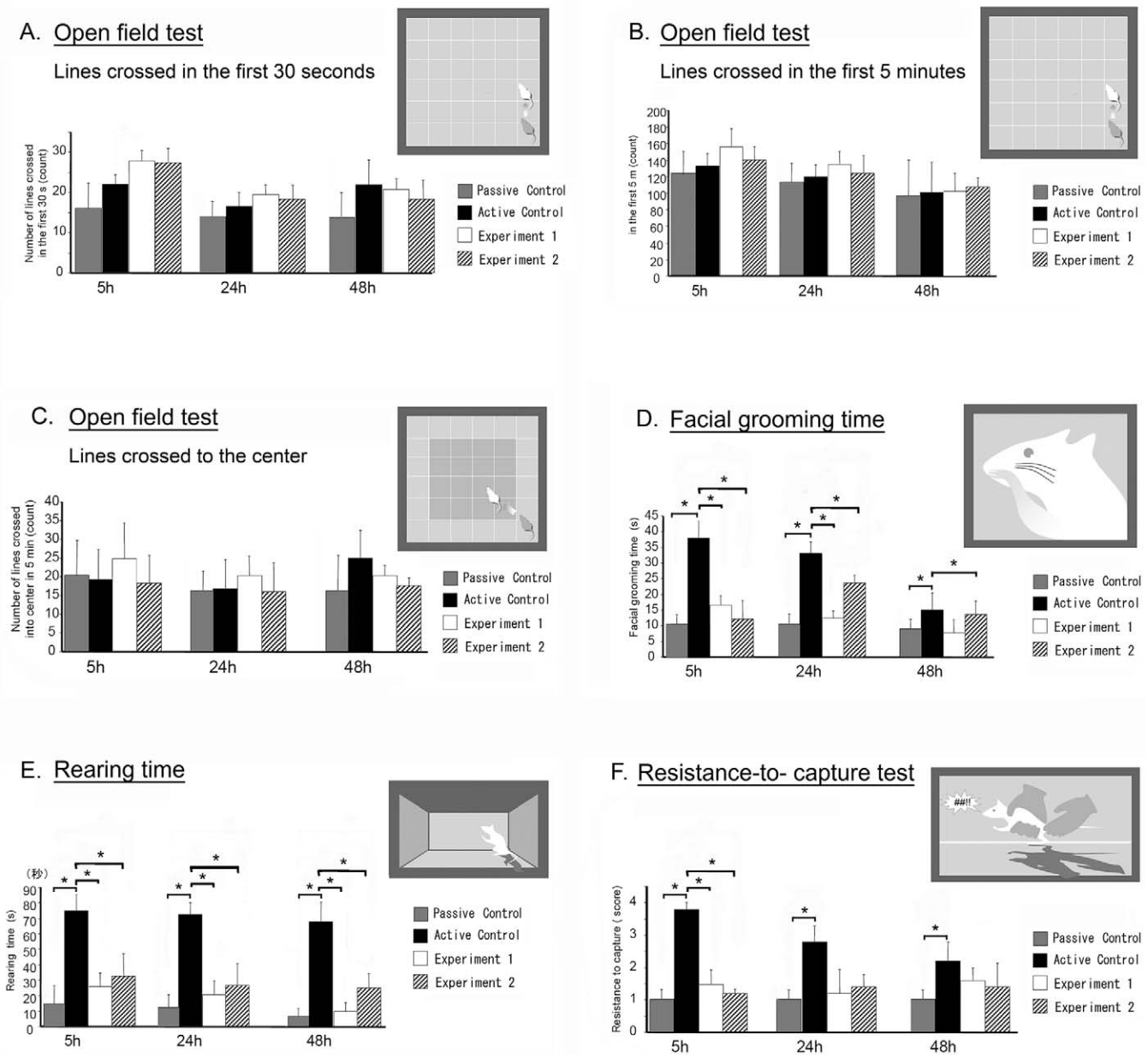

Figure 2. (A) Lines crossed in the first 30 seconds, (B) lines crossed in the first 5 minutes, and (C) lines crossed to the center were evaluated in the open field test. (D) Facial grooming time. (E) Rearing time. (F) Resistance-to-capture test.

the mean body weight decreased slightly, from 289.7 ± 10.3 g to 280.5 ± 17.0 g. In the active control group, the mean body weight decreased slightly, from 289.2 ± 12.4 g to 283.7 ± 14.1 g. In the experimental groups, the initial weight decreased from 290.0 ± 18.0 g before surgery to 285.2 ± 9.7 g at the end of the experiments.

The activity during the first 30 seconds of the 5-minute open-field test showed a tendency for the experimental groups to be more explorative than the control groups (Figure 2A). Total ambulation during the 5 minutes of the testing period was also higher in the experimental groups compared to the control groups at 5 and 24 hours. However, at 48 hours, the lines crossed by all the rats were similar (Figure 2B). Likewise, analysis of the number of lines crossed into the central area showed that rats in experimental group 1 went into the central area more often than those in experimental group 2 and in the control groups (Figure 2C). Facial grooming time was the shortest in the passive control group and experimental group 1 (celecoxib 30 mg/kg) throughout the experimental time (Figure 2D). Likewise, rearing time in the open field was less in the experimental groups. Compared with the passive control and the experimental groups, there were statistically significant differences at 5 hours, 24 hours,

and 48 hours for the total duration of the experiment in the active control group (Figure 2E). The resistance-to-capture test scores of the active control group received significantly higher scores compared to the passive control and experimental groups, especially at 5 hours. Experimental groups 1 and 2 showed values similar to those of the passive control group (Figure 2F).

DISCUSSION

In the present study, there was significantly more facial grooming activity in the active control group compared with the passive control group, and the increased activity was almost diminished in the groups treated with celecoxib. Excessive facial grooming in rats is a characteristic behavior indicative of orofacial pain.[17] Therefore, the remarkably reduced facial grooming activity observed in experimental group 1 may indicate that the pain was evoked by the mechanical force exerted from the activated spring on the periodontal ligaments and was effectively suppressed by celecoxib.

Preemptive administration of celecoxib reduced pain, as demonstrated by the lower pain and stress scores in the experimental groups. These scores were close to those of the passive control group. Of the two experimental groups, the one that received celecoxib before every behavioral test showed the lower values. This demonstrates that one preemptive dose administered before the appliance set, reinforced by two more drug administrations, resulted in less pain and discomfort in the rats. However, the results in experimental group 2 (a single dose of celecoxib 90 mg/kg before the appliance set) were not statistically significantly different from those of experimental group 1. The results suggest that the use of celecoxib 1 hour before force application may optimize analgesia and improve orthodontic care.

The ambulation observed in the rats on the second day, as evaluated by the lines crossed into the center, was different in the experimental and the active controls. This emphasizes the higher level of stress and anxiety in rats in the active control group 2 days after appliance placement.

With regard to the resistance-to-capture test, the active control group showed significantly higher scores, compared to the passive control and experimental groups, especially at 5 hours after the appliance was set. This result indicates that rats with an active appliance that did not receive pharmacologic treatment were experiencing a higher level of stress. Stressed rats are more likely to respond aggressively to being picked up by an examiner[18] and thus receiving higher scores. In previous studies, extremely stressed rats generally showed a mean resistance-to-capture score of 5, whereas intact rats usually showed a mean resistance-to-capture score around 1[19]; rats with occlusal disharmonies showed increased levels of stress hormones, peaking at about 6.5 to 8.5 hours.[20] In our study, rats in the active control group showed the highest scores compared to the rest of the groups. These scores gradually decreased with time. The passive control group showed the lowest score (about 1), whereas the experimental groups showed mean scores above 3 on the first day; these scores remained above 2 throughout the experiments. This tendency suggests a difference in the stress response between groups as early as 5 hours after placement of the appliances and may indicate that the acute pain is closely related to the higher scores, as in the active appliance non-drug group (active control). The differences in stress-related behavior among the groups might have been a result of at least two factors. First, rats were stressed from the pain induced by orthodontic force. Second, the stress caused by pain was relieved by administrations of anti-inflammatory medicine.

Further evaluations including the dose of drug, frequency, period, and tooth movement are necessary to establish a pain control protocol that is applicable to orthodontic patients.

CONCLUSIONS

- Administration of celecoxib 1 hour before setting an orthodontic appliance followed by 2 days of drug intake reduced the levels of pain and discomfort produced by orthodontic treatment in rats, as evidenced by pain-related behaviors.
- The present animal model might be a useful tool for the evaluation of pain and stress evoked by orthodontic force.

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