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Original Article

A modification of a previous model for inflammatory tooth pain: Effects of different capsaicin and formalin concentrations and ibuprofen

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Abstract

BACKGROUND AND AIM: This study aimed to solve the problems faced with the previous model of inflammatory tooth pain in rats.

METHODS: After cutting 2 mm of the distal extremities, the polyethylene crowns were placed on the mandibular incisors. In contrast to the original model, we used flow composite instead of wire in order to maximize the retention of crowns. Different concentrations of capsaicin (10, 25 and 100 mg/ml) and formalin were administrated into the cavities under the crowns. The algesic agent-induced behaviors were evaluated.

RESULTS: The modified model had no liquid leakage. Furthermore, composite allowed the crowns to remain for a longer period of time. Capsaicin 25, 100 mg/ml and formalin applications induced significantly more painful stimulation compared with control groups ($P < 0.001$). These responses were significantly reduced by the administration of ibuprofen, 20 minutes prior to the capsaicin 100 mg/ml injection.

CONCLUSIONS: This model seems to be adequate for long-term pain related experiments in which fluid leakage elimination is important.

KEY WORDS: Odontalgia, Capsaicin, Formalin, Model, Rat

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Painful conditions are highly important health concern as well as increasingly researched topic of study.[1,2] Pain activates a wide range of cortical and sub-cortical structures. For example, spontaneous firing of neurons in the primary somatosensory cortex and the ventral posterior medial nucleus of the thalamus induces a wide range of compounds affecting different neurotransmitter systems.[3] Orofacial pain is the most prevalent pain that people are afflicted with and odontalgia is the most commonly experienced type.[1,4] Because of the clinical significance of pain, emphasis should be placed on pain research. In an early review, Beecher cited 60 original publications in 1957 that were related to the description, development, and application of experimental tests of pain in animals.[5] By 1999, more than 425 reports were published in these regards.[6] This certainly reflects the heightened interest in understanding the mechanisms and side effects of pain.

Animal models have been used widely in basic pain research to investigate the potency and efficacy of the pharmacologic action and the molecular response to new agents.[2,6] Although a variety of pain models have been developed, very few odontalgia models are available. Most of the studies have applied the electrical tooth stimulation methodology to investigate the nociception in dental pulp. Despite various advantages, it is not a natural type of stimulus like those encountered by an animal in its normal environment.[6] More importantly, intense electrical stimuli excite

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all peripheral fibers, including large diameter fibers, which are not directly implicated in nociception. Brief and sudden electrical stimulation of dental pulp may produce highly synchronized neural signals and lead to wide behavioral responses.[6] Chemical stimuli are clearly different from electrical stimuli in regards to sensory and signal transduction as well as pattern of conductivity. They simulate features of human pain more closely. Typical reflexes, which necessitate a minimum level of synchronization of activity in primary afferent nerves, are inhibited by these stimuli. Moreover, drug release can be controlled in this way.[7,8]

Given the general consensus that current animal models of pain are suboptimal, it is important to consider what can be done to improve them.[6,9,10] In 2002, Chidiac et al. developed a new dental pain model induced by chemical inflammatory agents applied to rat incisors.[11] Although many advantages can be cited with respect to the use of this model, practical problems have prevented it from being widely used.

For example, in a pilot study, it was observed that the rats used their forepaws to remove the wires and crowns used in Chidiac's Model. Consequently, loss of crown retention and liquid leakage were found to be the dominant problems regarding long-term experiments. In this study, we aimed at improving and validating the above mentioned Chidiac's Model. We followed the time-course hypersensitivity of animals under these pain conditions and its reversal by ibuprofen as an analgesic drug.

Methods

Animals

Fifty-six adult male Wistar rats weighing 250-300 g were provided by the Neuroscience Research Center, Kerman University of Medical Sciences, Iran. Animals were kept on a 12-hour day-night schedule (lights on at 7:00 am) under standard laboratory conditions (temperature: 23 ± 2° C; relative humidity: 40%–50%) with standard rat chow and water ad libitum. All experimental procedures were approved by the Animal Research Ethics Committee of Kerman University of Medical Sciences, Kerman, Iran (Code: K/90/258).

Dental procedures

Animals received intraperitoneal (IP) injections of xylazine (Alfasan, Woerden, Holland) and ketamine (Alfasan, Woerden, The Netherlands) mixture (3 mg and 78 mg per kg bodyweight, respectively). A retractor was used to keep the animal's mouth open and the tongue to the side. The distal 2mm of the mandibular incisors were cut off using a fissure bur (Diatech, Heerbrugg, Switzerland) with a high-speed hand piece and copious water spray. Great care was taken not to expose the pulp.

Special crowns were designed to specifically fit over the incisors and mimic the natural occlusion as closely as possible. Crowns were made of polyethylene plastic except for the coronal plane, which was sealed by a metal cap that was fully covered with cyanoacrylate adhesive (Super Bonder; Loctite Brasil Ltda, Itapevi, SP, Brazil). Five auxiliary retention holes were placed (2 in the buccal aspect and 1 in the lingual and lateral aspects) using a slow-speed round bur (Fig. 1).

Figure 1. The artificial crown design

Teeth were acid etched (Kimia, Tehran, Iran) on all surfaces, except for cut edges. After applying bonding agent (Heliobond, Ivoclar-Vivadent, Liechtenstein) and polymerization, the teeth surfaces were

covered with tetric flow composite (Tetric Flow, Ivoclar Vivadent) and crowns were placed on the teeth. The auxiliary holes were filled automatically as to increase the retention of the crowns. A small space (hallow chamber) remained between the tooth structure and the internal surface of the crown (Fig. 2). Chlorhexidine 0.12% (Sharedaru Pharmaceutical Co., Tehran, Iran) was applied topically to the gingiva around the crown twice daily with a cotton swab.

Figure 2. Polyethylene crowns placed onto mandibular incisors using Tetric Flow, a flowable resin composite. A small cavity was left between the metal cap and the cut end of the teeth.

Study drugs

Formalin 2.5%: Formalin solution was freshly prepared from commercially available stock formalin (Sigma-Aldrich) diluted in isotonic saline to 2.5%. Stock formalin is an aqueous solution of 37% formaldehyde.

Capsaicin (Sigma-Aldrich): Capsaicin was dissolved in Tween 80 (Merck, Germany)-ethanol solution (10% ethanol, 10% Tween 80, 80% distilled water, w/w) at the graded concentrations of 10, 25 and 100 mg/ml and administrated intradentally (i.d.).

Ibuprofen (Kimidaru, Iran): Ibuprofen powder with vehicle (2% Tween 80/distilled water) in a dose of 120 mg/kg was administered by oral gavage.

Study groups

Fifty-six animals were randomly divided into eight groups (N = 7) as follows:

1: Control group (CO) included intact animals.

2: Sham operated group (SO) received i.d. injection of normal saline.

3: Sham vehicle group (SV) received i.d. injection of vehicle of capsaicin including Tween 80 and ethanol.

4-6: Capsaicin treated groups (C10, C25 and C100) received i.d. injection of 10, 25 and 100 mg/ml capsaicin, respectively.

7: Formalin treated group (F) received i.d. injection of formalin 2.5%.

8: Ibuprofen treated group (I) received ibuprofen 20 minutes before i.d. capsaicin 100 mg/ml.

After two days of recovery, unanesthetized rats were restrained in plastic holding tubes and the mouth was held open with the use of a small retractor. According to the study group, 10 µl of the specified drug was injected in the hallow chamber through a 27-gauge needle as quickly as possible and cyanoacrylate adhesive was used to close the crown perforation immediately. 4x magnifying loops were utilized.

Moreover, intradental injection of methylene blue dye was used to evaluate the sealing ability of the crowns in six rats, three with wire retention crowns and three with composite retention crowns.

Nociceptive behavior

Test sessions were carried out during the light phase between 10:00 and 17:00 in a quiet room maintained at 23–24° C. Before the injection, each animal was placed in the test box for a 30-min habituation period to minimize additional stress. The rats did not have access to food or water during the test.

Immediately following the injection, each rat was placed back in the transparent Plexiglas box (25 cm × 35 cm × 35 cm) with a transparent floor positioned over a mirror at the angle of 45 degrees to allow the

observation of nociceptive behavior. The behavior of the rats was observed for 21 minutes. The recording time was divided into 7 blocks of 3 minutes. A pain score was determined for each block by measuring the number of seconds that the animal presented each of the following responses (the same scoring criteria as Chidiac et al. study).[11] Zero indicated calm and normal behavior, including grooming; 1, abnormal head movements including mild head shaking or continuous placement of the jaw on the floor or the wall of the cage; 2, abnormal continuous shaking of the lower jaw; 3, excessive rubbing of the mouth with foreleg movements, such as head grooming, but concentrated consistently and mainly on the lower jaw. A video camera was used to record the behavioral response. Upon application of deep anesthesia, all animals were sacrificed at the end of the observation period.

Statistical analysis

Analysis of the nociceptive behavior was completed by an investigator who was blinded to the animal's group assignment. Behavioral data comparing the different groups over the total period of testing was analyzed by means of two-way repeated ANOVA measures followed by Tukey's post hoc test.

Results

The pilot study revealed that the animals exhibited an immediate nociceptive response to formalin and capsaicin injections, with a marked peak in the 18-21 minutes. In the control group, animals suffered no pain.

Normal saline values were not significantly different from those observed in control animals.

Capsaicin 25, 100 mg/ml and formalin applications induced significantly more painful stimulation compared with the SV and SO groups ($P < 0.001$). In contrast, administration of ibuprofen 20 minutes before the capsaicin 100 mg/ml was associated with a significant decrease in pain scores similar to that observed in the control group (Fig. 3).

Capsaicin 100 mg/ml treated animals spent a significantly higher amount of time in pain score 2 and 3 compared with C10 and I groups. The greatest effect was associated with capsaicin 100 mg/ml application (Fig. 4). Crowns cemented with composite revealed no visible leak of methylene-blue dye. In contrast, extensive leakage was observed in wire retention crowns.

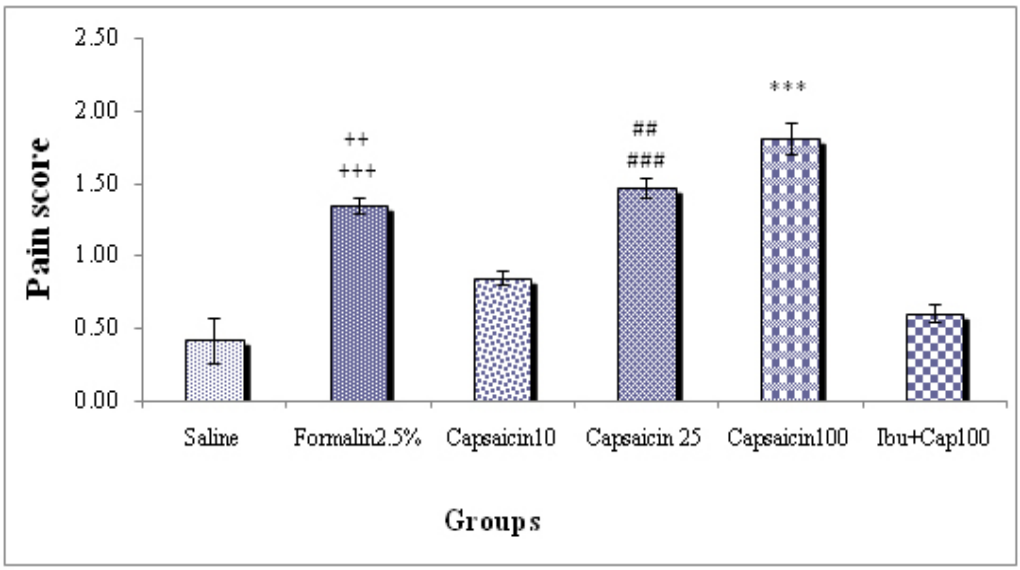

Figure 3. Pain scores recorded in different study groups

*** P < 0.0001 vs. formalin, capsaicin 10 mg/ml, ibuprofen + capsaicin 100 mg/ml and saline
+++ P < 0.0001 vs. capsaicin 100 mg/ml and sham ++ P < 0.01 vs. capsaicin 10 mg/ml
P < 0.0001 vs. capsaicin 10 mg/ml ## P < 0.01 vs. capsaicin 100 mg/ml

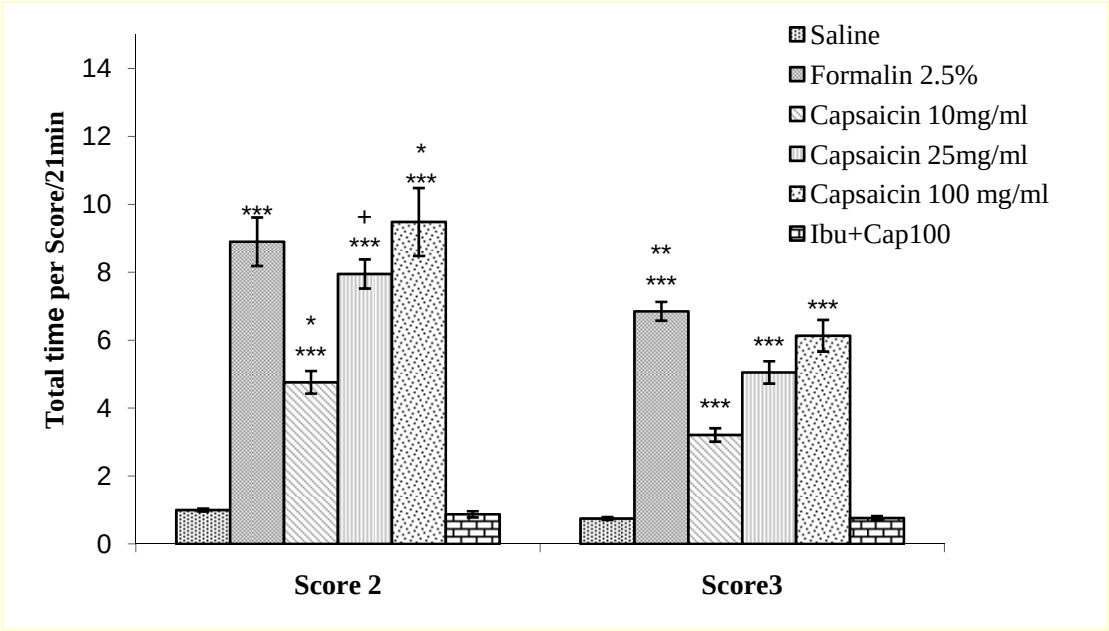

Figure 4. Total time spent in score 2 and 3 for different study groups

Score 2
*** P < 0.0001 vs. Saline and Ibuprofen + capsaicin 100 mg/ml
* P < 0.05 vs. capsaicin 25 mg/ml
+ P < 0.05 vs. capsaicin 10 mg/ml

Score 3
*** P < 0.0001 vs. saline and Ibuprofen + capsaicin 100 mg/ml
** P < 0.01 vs. capsaicin 25 mg/ml

Discussion

In the current study, we introduce an optimized behavioral model to study inflammatory tooth pain conditions by characterizing the nociceptive behavioral responses induced by the intradental injection of capsaicin and formalin.

Pain experimentation on human subjects is practically challenging, fundamentally subjective, and ethically self-limiting. Given these restraints, laboratory animal models of pain are widely used.[12] Gabka and Price found that, as a pain model, tooth pulp stimulation provided both repeatable results and good correlation between experimental and clinical analgesia.[13] Numerous investigators have described morphologic similarities between human and rat teeth.[14,15] It has been shown that the physiology and histology of human and rat pulps is probably similar.[16] Current animal models of pain are suboptimal and need to be improved. Proposals for improvement can be grouped into several categories including refinement of current models to enhance their accuracy and reduce their variability as well as the development of new models.[17]

Recently, Chidiac et al. introduced a new, intriguing model for inflammatory tooth pain.[11] In their model, artificial crowns were fixed on the incisors of rats by an orthodontic stainless steel wire. In a pilot study, we observed that the rats used their forepaws to remove the wires and crowns. As a result, loss of crown retention and liquid leakage were found to be the dominant problems for long-term experiments. Here, we presented a modified tooth pain model that can be widely used to study different fields of pain including the mechanisms as well as to test the effects of analgesics, especially at the peak of pain. Through our optimized model, improved crown retention and seal ability is achieved after providing retention auxiliary holes and using flow composite instead of wire.

The present study demonstrates that the injection of formalin rather than saline into the crown cavities produces quantitative nociceptive behaviors. This suggests that the pain behavior was not due to increased pressure and/or volume expansion because of the injected drug. A similar inefficiency of

saline in inducing nociceptive behaviors has already been noted in the temporomandibular joint (TMJ) region of rats[18], in the paw[19] and in the upper lip formalin test.[20] This was also demonstrated in the study by Chidiac et al. which mentioned that i.d. application of saline did not influence nociceptive scores.[11]

A number of mechanisms have been postulated to explain the formalin-induced orofacial pain in rats. Formalin predominantly evokes activity in C fibers,[21] which are found in the subodontoblastic layer and the deep pulp. The initial response is derived from direct chemical stimulation of nociceptors resulting in C-fiber firing through TRPA1 channels; however, ongoing inflammatory input and central sensitization causes the next step.[22]

One of the characteristics of the formalin response is its biphasic pattern.[21,22] However, the i.d. injection of formalin in the present study, demonstrated just one response phase. This is in line with the Roveroni et al. study which showed that the injection of formalin into the TMJ region induced only one pain phase.[18]

Since the formalin related nociceptive behavior is concentration-dependent,[20] we considered 2.5% formalin based on previous studies,[11,20] which indicated that formalin responses reached a maximum at 2.5%.

In the present study, Injection of capsaicin produced a dose-related pain response. Although the exact mechanism of how capsaicin elicits the sensation of pain upon the nociceptors is still not completely clear,[23] there are several proposed theories in this regard. Capsaicin, the primary pungent ingredient in hot chili peppers, has a selective action on small sensory fibers that convey pain sensations and elicit axon reflex vasodilatation.[24] Excitotoxic action of capsaicin has been shown on spinal afferent neurons expressing vanilloid receptor subtype 1 (VR1).[25] Functionally, VR1 is a nonselective cation channel that displays an exceptionally high permeability for Ca2+ and is expressed on a major subclass of nociceptors, including unmyelinated C fibers and some lightly myelinated A-delta fibers.[26] Pulpal C nociceptors are thought to have a predominant role in encoding inflammatory pain arising from dental pulp and periradicular tissue. This intriguing aspect of VR1 is likely to explain the burning sensation of capsaicin-evoked pain.[27] Capsaicin injection into the orofacial region also simultaneously increases the spontaneous firing of neurons in the primary somatosensory cortex and the ventral posterior medial nucleus of the thalamus.[28] In addition, activation of capsaicin-sensitive fibers in dental pulp increases pulpal blood flow. It has been reported that topical application of capsaicin in the maxillary region of the monkey produces changes in escape behavior, suggesting a thermal and mechanical hyperalgesia.[29]

Ibuprofen resulted in a significant decrease in the response score, which was comparable to that of the saline values, when it was given prior to capsaicin 100 mg/ml administrations. In contrast, Brandt reported that ketorolac as a non-steroid anti-inflammatory drug did not prevent hypersensitivity produced by capsaicin.[30] Moreover, in a study by Jones et al. ibuprofen had weak efficacy in attenuating capsaicin-induced mechanical allodynia in rats.[31] The difference could be attributed to the type of drug used, the dosage, route of administration and the factors associated with anatomical location of sensory endings.

It is noteworthy that, compared with placement of the jaw on the floor, continuous shaking of the lower jaw and excessive rubbing of the mouth were strongly correlated with capsaicin concentration. Although the behavior of rubbing the orofacial region resembles that of washing the face, prolonged face rubbing is not displayed spontaneously by normal intact rats.[32] This data demonstrates that mandible movement and rubbing behaviors are more related to pulpal pain, rather than placement of the jaw on the floor. On the other hand,

grooming was a common occurrence, even in intact animals, and resembled a general normal reaction rather than a pain response. Based upon this information, we assigned no score to this behavior.

There are some potential limitations of our study. After the surgical intervention, the animals' food intake was switched from pellets to powder in order to prevent weight loss. These stresses due to environmental changes may interfere with the pain process at the central and peripheral level. Furthermore, animal restriction followed by i.d. injection of the drug may result in an uncomfortable position, making it difficult to see the animals' normal responses to a stimulus. Finally, since the cerebral cortical structures participate in the conscious perception of pain,[3] it is imperative that assessments of pain in laboratory animals quantify behavioral responses to sensory experiences that are cortically medicated. Humans possess certain neuroanatomical features crucial for pain sensation that are not present in some of the species that most commonly used in pain research, such as rodents.

Conclusion

In summary, our findings suggested that the described experimental approach is a valid model of inflammatory dental pain. We concluded that intradental capsaicin or formalin-induced nociceptive rubbing and mandible movement responses may be used as indexes of inflammatory tooth pain.

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Conflict of Interest

Authors have no conflict of interest.

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