Analgesic Effectiveness of Ketorolac Compared to Meperidine in the Rat Formalin Test

Bryan C. Randolph, DDS, MS, and Marvin A. Peters, PhD
School of Medicine, Department of Pharmacology, Loma Linda University, Loma Linda, California

The rat formalin test is an analgesic behavioral observation assessment method that demonstrates two phases of nociceptive behavior. The test consists of injecting the right hind paw with a 5% formalin solution and then observing the animal for specific nociceptive behavior. The phases represent two different types of pain. Phase 1 is pain produced by direct nerve stimulation and phase 2 is an inflammation-induced pain. The nociceptive behavior measured in this experiment was licking and biting the injected paw. A comparison of nociceptive behavior was made when ketorolac and meperidine were injected (ip) 10 min prior to formalin injection. As expected, a biphasic pattern of licking and biting the injected paw ensued. It was found that ketorolac had no significant reduction in licking and biting, while meperidine dramatically reduced the nociceptive response in phase 1. In phase 2, both ketorolac and meperidine caused a reduction in licking and biting; however, meperidine reduced the nociceptive response to a greater extent. This experiment demonstrates that ketorolac, when compared to meperidine, is less effective in treating pain from inflammatory origin and is not effective in treating pain from direct nerve stimulation.

Key Words: Formalin test; Ketorolac; Meperidine; Analgesia; Inflammation; Nociception.

The formaldehyde (formalin) hind paw test[1] used in this study is an analgesic behavioral observation assessment method used to measure the effectiveness of antinociceptive agents. The advantage of the formalin test over other methods that measure nociception is that two different types of pain may be evaluated over a prolonged period of time and the test thus allows analgesics with different mechanisms of action to be compared. This is in contrast to other methods such as the hot plate test,[2,3] which has been found to be relatively ineffective in evaluating analgesics of the nonsteroidal anti-inflammatory type[4] but does measure the effectiveness of opioid-type analgesics.

The test consists of injecting the hind paw with formalin and then observing the animal for nociceptive behavior in the form of licking and biting the limb. It has consistently been found that two distinct phases of licking and biting occur[5]: phase 1—a short but immediate response lasting the first 5 min after the hind paw is injected; phase 2—a prolonged response starting at approximately minute 11 and ending at about minute 50. Between phases 1 and 2, there is an intermittent period from minute 6 to minute 10 where little nociceptive behavior is observed. It is theorized that the two different phases represent two qualitatively distinct types of pain. Phase 1 is a direct stimulation of the nerve by the formalin and phase 2 is an inflammatory reaction-induced pain.[6] When administered to humans, 5% formalin causes immediate pain, described as intense, sharp, stinging, and burning. After 4 to 5 min, this gives way to a steady, throbbing ache, which gradually disappears over 30 to 60 min.[7] It has been found that opioids such as morphine reduce pain behavior in phase 1 and phase 2, while nonsteroidal anti-inflammatory agents such as indomethacin have little if any effect on phase 1 but reduce the nociceptive behavior of licking and biting in phase 2.[8] Therefore, by objective observation of the animal's behavior in phase 2, maximum efficacy may be

Received June 10, 1996; accepted for publication March 6, 1997.
Address correspondence to Dr. Marvin A. Peters, Loma Linda University, School of Medicine, Department of Pharmacology, Loma Linda, CA 92350.

Anesth Prog 44:11–16 1997

ISSN 0003-3006/97/$9.50
SSDI 0003-3006(97)

obtained for both nonsteroidal antiinflammatory agents (NSAIDs) and opioid agents. The mechanism of action of NSAIDs is the inhibition of cyclooxygenase 2,[9,10] and opioids are working by modulation of opioid receptors in the central nervous system.[11] The purpose of this study was to compare the efficacy of ketorolac and meperidine using the rat formalin test.

Ketorolac tromethamine was approved by the FDA on November 30, 1989, for the treatment of short-term pain.[12] A number of clinical studies have evaluated ketorolac in postoperative pain and found it to be equal or greater in effectiveness than meperidine.[13–17] Therefore, it was hypothesized that there would be no difference between ketorolac and meperidine regarding maximum efficacy in phase 2.

METHODS

Male Sprague-Dawley rats weighing 250 to 399 g were used. The animals were housed in shoebox-type cages and placed in a climate-controlled room (temperature 68 to 72°F, humidity 49 to 52%) with 12-hr day/night cycle (6:00 a.m. to 6:00 p.m.). Food and water were available *ad libitum*. Animals were purchased from Charles River Laboratories and allowed to acclimate in the previously described environment for a minimum of 24 hr before the experiments were started. This experiment was carried out in compliance with the institutional animal-care guidelines of Loma Linda University. All medicinal agents used in this experiment, including saline, were administered by intraperitoneal (ip) injection. The control group, consisting of eight animals ($N = 8$), was given 0.5 ml of 0.9% sodium chloride (saline). The experimental group of ketorolac was divided into five subgroups: 1.1, 2.3, 4.5, 10.2, and 20.3 mg/kg. The experimental group of meperidine was also divided into five subgroups: 3.8, 5.7, 8.5, 12.8, and 19.2 mg/kg. Each subgroup of both ketorolac and meperidine consisted of eight animals ($N = 8$). The concentration of the agents were adjusted with 0.9% NaCl so that each animal in every group received a total volume of 0.5 ml of solution. These dosages were chosen based on extrapolation from previous experiments[18] and a pilot study.

The following agents where used: pentobarbital sodium injection, USP (Abbott Laboratories, North Chicago, IL 60064), meperidine hydrochloride, USP (Sanofi Winthrop Pharmaceuticals, New York, NY 10016), 0.9% sodium chloride, USP (Abbott Laboratories, North Chicago, IL 60064), halothane, USP (Halocarbon Laboratories, River Edge, NJ 07661), ketorolac tromethamine, USP (Hoffmann-La Roche Inc., Nutley, NJ).

Ten min after ip administration of 0.9% sodium chloride, meperidine HCL, or ketorolac tromethamine, the animal was anesthetized with 1 ml halothane deposited on a 4 × 4-inch gauze pad and then placed over the nose and mouth of the animal. Immediately after the animal lost consciousness, the halothane was removed and the right hind paw was injected with 0.05 ml of 5% formalin in saline subcutaneously using a 27 gauge ½-inch needle. The animal was placed in a shoebox-type cage next to a mirror so that the animal could be observed from all angles. All animals regained consciousness within 30 to 60 sec and began to roam the cage. The animals were continuously observed from the time of regaining consciousness to 50 min. The behavior of the animals was recorded by manually entering data into a computer program that tabulated the number of seconds per minute the animal spent licking and biting the injected foot. The time spent licking and biting was monitored continuously and recorded as seconds per minute from minute 1 through minute 50. Paired *t*-tests were performed for statistical analysis with use of StatView®[19]. The tests compared the saline control with the treatment groups. Significance was considered achieved at $P \leq 0.0500$. After the experiment was completed, all animals were euthanized with 200 mg/kg pentobarbital sodium ip.

RESULTS

Two parameters of nociceptive response measured in this study were licking and biting. The data obtained for the saline control (Figure 1) showed a biphasic nociceptive response with an immediate and short burst of activity lasting approximately the first 5 min (phase 1), followed by a prolonged period of activity (phase 2) starting at minute 11, peaking between 20 to 30 min, and subsiding by 50 min after the injection. Little nociceptive behavior was observed during a 5-min intermittent period from minute 6 to minute 10. The ineffectiveness of ketorolac on phase 1 of the pain response is shown in Figure 2. None of the doses of ketorolac consistently or significantly affected the nociceptive response. In contrast, meperidine significantly and consistently reduced the nociceptive response in phase 1 (Figure 3). Every dose of meperidine reduced licking and biting until virtually dissipated. Clearly, meperidine was effective in reducing the response produced in phase 1 of the formalin test.

In phase 2 pain, ketorolac produced a significant reduction in nociceptive response (Figure 4). The maximum reduction in licking and biting was limited to approximately 25% of that for the saline control. This 25% reduction occurred at a dose of 4.5 mg/kg ketorolac, and larger doses did not further decrease the re-

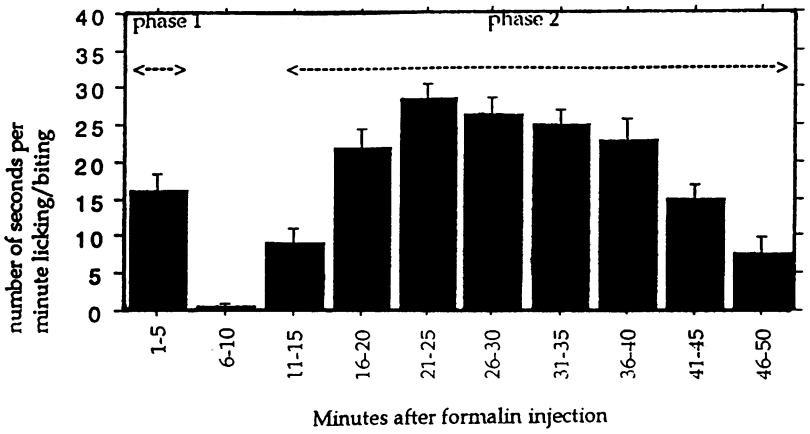

Figure 1. Response to pain (licking/biting) in the saline controls. The data has been averaged over 5-min time periods and is presented as the mean + 95% confidence interval for eight animals.

sponse to formalin. Although effective, ketorolac is limited in its ability to attenuate the nociceptive behavior produced in phase 2. Meperidine, as did ketorolac, significantly reduced licking and biting in phase 2 (Figure 5). In contrast to ketorolac, the dose–response to meperidine was not limited, and the largest dose of 19.2 mg/kg greatly reduced the response. However, meperidine did not reduce the nociceptive response in phase 2 as much as it did in phase 1. Thus, meperidine is effective in both phase 1 and phase 2, while ketorolac is effective in phase 2 only.

DISCUSSION

The purpose of this study was to objectively assess the effectiveness of ketorolac and meperidine in treating two different types of nociception using the rat formalin test. Most reported studies used licking and biting as the measure of assessing nociception. Results of our studies with ketorolac are consistent with other studies using NSAIDs.[20] This provides evidence that ketorolac, like other NSAIDs, most likely relies on the inhibition of cyclooxygenase for its analgesic action. Meperidine, an opioid-type analgesic, modulates the perception of painful stimuli through interaction with opioid receptors in the central nervous system. Thus, it is effective in eliminating pain associated with direct nerve stimulation.

It has been shown that the nociception seen in phase 1 is a result of direct nerve stimulation by the formalin.[21] Meperidine administration caused a dose-related reduction in the licking and biting response during phase 1. The highest dose of meperidine resulted in virtual elimination of the nociceptive response. In phase 2, ketorolac caused significant reduction in the licking and biting response at low doses, with the maximum effect seen at 4.5 mg/kg. After a dose of 4.5 mg/kg ketorolac, no further reduction in nociceptive behaviour occurred as the dose was increased. Meperidine likewise had a statistically significant reduction in licking and biting response in phase 2. In contrast to ketorolac, how-

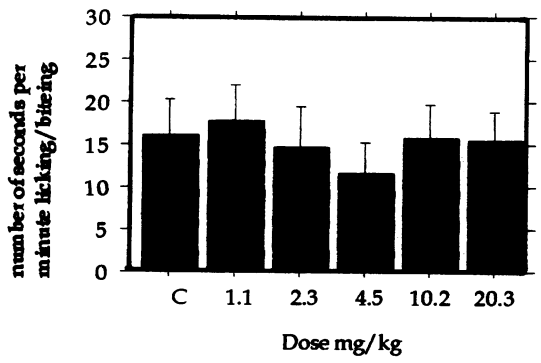

Figure 2. Mean dose response + 95% confidence interval for ketorolac in phase 1 of the pain response. The data presented represent minutes 1 through 5 for each dose.

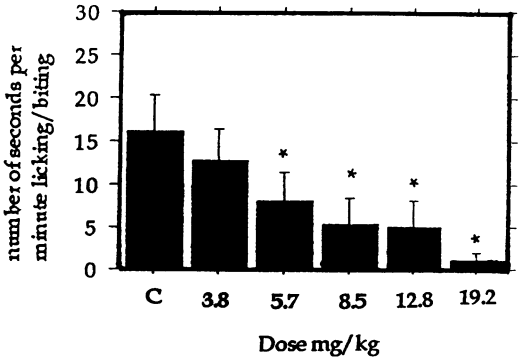

Figure 3. Mean dose response + 95% confidence interval for meperidine in phase 1 of the pain response. The data presented represent minutes 1 through 5 for each dose.*, $P \leq 0.05$.

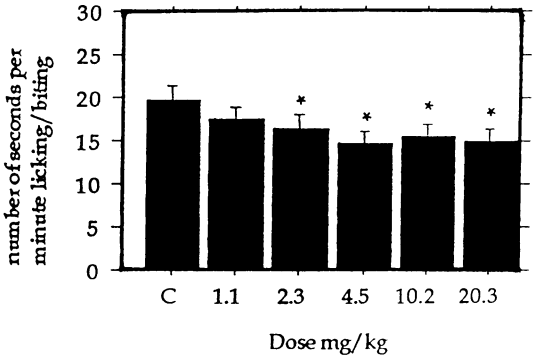

Figure 4. Mean dose response + 95% confidence interval for ketorolac in phase 2 of the pain response. The data presented represent minutes 11 through 50 for each dose.*, $P \leq 0.05$.

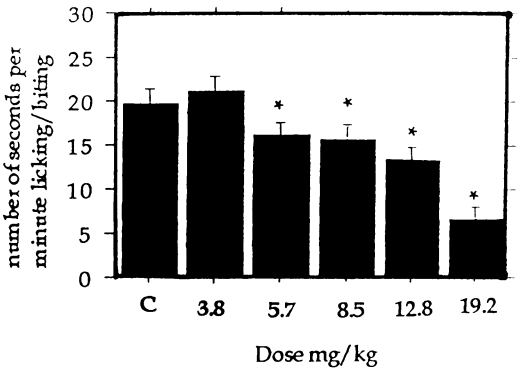

Figure 5. Mean dose response + 95% confidence interval for meperidine in phase 2 of the pain response. The data presented represent minutes 11 through 50 for each dose.*, $P \leq 0.05$.

ever, no maximum analgesic ceiling was reached with meperidine in phase 2.

It has been demonstrated that the nociception produced in phase 2 of the formalin test is a result of chemical insult resulting in tissue damage.[22] Tissue destruction produces mediators of inflammation such as histamine,[23,24] bradykinines,[25] prostaglandins,[26–28] and serotonin.[29] Ketorolac, an NSAID, blocks the production of prostaglandins[30]; therefore, sensitization of the peripheral nervous tissue is reduced, resulting in less nerve stimulation and ultimately less pain. In addition to the peripheral activity, it has been proposed that significant modification of central system neurons occurs. There is a reduced threshold of dorsal horn neurons to stimulation triggered by inputs from afferent neurons,[31–33] an expansion of the receptive fields of dorsal horn neurons,[34,35] and a summation of slow postsynaptic potentials, resulting in a cumulative depolarization and a prolonged after-discharge of dorsal horn neurons. The latter is referred to as "windup."[36] Studies have been ongoing to elucidate the compounds involved in this central nervous system phenomena and include N-Methyl-D-Asparate antagonists and amino acids.[37] Opioid analgesics such as meperidine may be involved with modulating this process, in addition to their other known central nervous system mechanisms of action.

Both ketorolac and meperidine are analgesic in phase 2 of the formalin test. This allows comparison between these two classes of agents as far as analgesic efficacy is concerned. Our data show that the efficacy of ketorolac is limited in phase 2 compared to meperidine and that ketorolac is not effective in phase 1. These data support the hypothesis that ketorolac is limited in the type of nociception it can be used to treat and that, even in the inflammatory type of pain where it has its effects, the maximum efficacy is limited compared to meperidine, an opioid analgesic.

Many clinical studies have measured the effectiveness of ketorolac tromethamine. In dental practice, it was found to be effective in reducing pain after third molar tooth removal[15] and in severe odontogenic pain relative to a placebo control.[38] In similar studies, the analgesic effectiveness of 30 and 90 mg ketorolac intermuscular (im) was greater than 50 and 100 mg of meperidine im in postoperative pain from impacted third molar tooth removal.[13]

Ketorolac is also an equally effective and longer-acting analgesic than meperidine in postoperative pain from major surgery such as cholecystectomy, laminectomy, abdominal hysterectomy, and open reduction and fixation of fractures.[14] Thirty mg of ketorolac also has efficacy similar to 100 mg of meperidine in postoperative pain after major abdominal surgery.[39] These clinical studies have used subjective criteria such as visual analog scales and verbal response to measure the pain in order to rate the effect of the medication given.

The origin of postoperative clinical pain is thought to be a result of tissue destruction analogous to that produced in phase 2 of the formalin test.[40] Some of the discrepancy between the results obtained in our study and the clinical studies previously cited may be the subjective clinical responses to the effect of ketorolac and meperidine. In our study, specific behavioral licking and biting responses were measured and recorded. In clinical analgesic studies comparing ketorolac with placebo, the placebo effect alone accounts for approximately a 30% reduction in pain perception.[41,42] Because of the placebo response, subjective clinical analgesic studies may not be as accurate in estimating the therapeutic effectiveness of ketorolac. Also many, but not all, of the clinical investigations measure postoperative pain. Postoperative pain is likely caused by tissue injury that results in inflammation, producing nociception similar to that in phase 2 of the formalin test.[40] Since our study shows that ketorolac is effective in an inflammatory model, it

follows that ketorolac should be effective in clinical studies of inflammation-induced nociception. In our study, the maximum effectiveness was limited for ketorolac compared to meperidine. In the clinical studies cited, ketorolac was found to be equal to or even more effective than meperidine with the doses chosen for study. The clinical studies used a maximum dose of 100 mg meperidine. However, the maximum therapeutic dose of meperidine is approximately 150 mg in humans.[43] Therefore, the clinical studies used submaximal doses of meperidine. This may account for the difference in efficacy found in our study and the clinical studies, However, our study shows that ketorolac is limited in the type of nociception it is effective in treating and that its maximum effectiveness is limited compared to meperidine in an inflammatory model.

ACKNOWLEDGMENTS

We wish to thank the following individuals for their expert technical help and willingness to assist in this project: Jodi Reed, Jeff Sale, and Dave Gilsdorf of the Risley Hall Learning Center, School of Medicine, Loma Linda University, Loma Linda, CA. We also wish to thank Velma Clem, Daniel Cole, and Ralph Cutler, School of Medicine, Loma Linda University, Loma Linda, CA, for assisting in the preparation of this manuscript.

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