

Refinement of the dosage and dosing schedule of ketoprofen for postoperative analgesia in Sprague-Dawley rats

Dale M. Cooper, DVM, MS, Dipl. ACLAM¹, Wherly Hoffman, PhD², Kristi Tomlinson, LAT³ & Hsiu-Yung Lee, MS²

Though ketoprofen is commonly used in rodent surgical procedures, an optimal dosing regimen has not yet been established. The authors sought to refine ketoprofen dosage requirements in rats and to determine whether one or two doses were needed. In one experiment they compared the effects of one preoperative dose of ketoprofen with those of two perioperative doses (3 mg per kg body weight). In a second experiment they compared the effects of two different dosages of ketoprofen (3 or 5 mg per kg body weight). Results show that all regimens tested were similarly effective in curbing post-surgical weight loss and reduction in food and water consumption; therefore, a single dose of 3 mg per kg body weight was the most efficient.

The need for postoperative analgesics in rodents is well-established in animal welfare guidelines¹. Ketoprofen has been used by several investigators in studies of postoperative analgesic requirements and is commonly recommended by research institutions²⁻⁸. In both soft-tissue and orthopedic surgery, ketoprofen provides an analgesic effect similar to that of opioids such as buprenorphine. Unlike most opioids, however, ketoprofen has a long duration of action in many species, and only one daily dose is typically needed⁹. Additional options for analgesia in rodents include meloxicam and carprofen, but these do not seem to offer any specific advantages over ketoprofen^{4,8,10,11}. Long-acting local analgesics such as bupivacaine have not been shown to be effective in rodents¹².

The optimal dosing schedule for ketoprofen in rodents and the dose-response within the therapeutic range have not yet been conclusively established. Many different dosing regimens for rats have been evaluated in the literature and, in our experience, are recommended by major research institutions. Dosage can range between 3 mg and 20 mg per kg body weight and can be administered either parenterally or orally, as a single perioperative dose or up to three times daily^{2-8,13-15}.

There are data to refine this wide range of dosing recommendations. Our laboratory previously evaluated a regimen of two doses of 3 mg ketoprofen per kg body weight administered to rats before and after abdominal surgery. We showed that 24 h after surgery, there were differences in food and water consumption and body weight change between rats that received analgesic and those that received only saline. We did not observe differences 48 h after surgery². Another laboratory has consistently shown that for up to 6 h after abdominal surgery, a single perioperative dose of 5 mg ketoprofen per kg body weight reduces behaviors that seem to be indicative of post-surgical pain^{4,7,8}.

The purpose of our current study was to further refine dosage and dosing schedule recommendations for ketoprofen in rats by bridging the previous studies. It is desirable to use the lowest effective dose of ketoprofen and to administer it for as short an interval as possible in order to minimize the risk of side effects. These effects include intraoperative hemorrhage, which has been reported during rodent cannulation procedures at our institution, and gastrointestinal toxicity, which is a class effect for this type of drug¹⁶.

¹Veterinary Resources, Toxicology, ²Global Statistical Sciences, ³In Vivo Pharmacology Core, Eli Lilly and Company, Greenfield Laboratories, 2001 W. Main St., Greenfield, IN 46140. Correspondence should be addressed to D.M.C. (cooperd@lilly.com).

In one experiment we evaluated whether a single preoperative dose of 3 mg ketoprofen per kg body weight would be less efficacious in reducing post-surgical body weight loss or pain-related changes in food and water consumption compared with two perioperative doses of 3 mg per kg body weight. We took measurements 24 h after surgery. We chose to examine these parameters because they have previously been established as simple and reliable measures of the analgesic effects of ketoprofen and other analgesics. Additionally, these measurements can be taken without specialized monitoring equipment, which is needed for carrying out certain behavioral evaluations^{2–4,7,8,17–19}. We also thought that this method might detect evidence of pain that would not be detectable through behavioral monitoring. In a second experiment, we compared the effects of a single preoperative dose of either 3 or 5 mg ketoprofen per kg body weight on daily food and water consumption and body weight change.

METHODS

Study design

In the first experiment we used male Sprague-Dawley rats ($n = 40$) that were arbitrarily divided into the following four groups ($n = 10$ per group): (i) controls that did not undergo surgery; (ii) controls that underwent surgery but did not receive analgesia; (iii) rats that underwent surgery and received one preoperative dose of ketoprofen (3 mg per kg body weight); and (iv) rats that underwent surgery and received one preoperative and one postoperative dose of ketoprofen on the day of surgery (each dose was 3 mg per kg body weight).

In the second experiment we divided male Sprague-Dawley rats ($n = 30$) into three groups ($n = 10$ per group). We assigned rats to groups by stratified randomization according to presurgical body weight. The groups were as follows: (i) controls that did not undergo surgery and received a low dose of ketoprofen (3 mg per kg body weight); (ii) rats that underwent surgery and received a low preoperative dose of ketoprofen (3 mg per kg body weight); and (iii) rats that underwent surgery and received a high preoperative dose of ketoprofen (5 mg per kg body weight).

For both experiments we measured food weight, water volume and body weight at approximately 7:30 AM each day on the day before surgery, on the day of surgery (before the procedure) and on the day after surgery.

Rats

All activities were performed humanely under a protocol approved by our IACUC in a facility accredited by AAALAC, International. We used male 2-month-old Hsd: Sprague Dawley SD rats (Harlan, Indianapolis, IN) that weighed approximately 250–300 g each. We housed rats individually in appropriately sized shoebox cages on corn-cob bedding (bed-o-cobs, Maumee, OH). We fed them Lab Diet 5002 Certified Rodent Diet (PMI Nutrition

International, St. Louis, MO) and gave them 5- μ m filtered municipal tap water *ad libitum*. We maintained rooms at a temperature of $22 \pm 2^\circ\text{C}$ with 30–70% relative humidity, with 100% conditioned outside air that was changed 10–12 times per h. We used fluorescent lighting with a light:dark cycle of 12 h:12 h (lights on at 6:00 AM). Room sentinels were tested quarterly and were free of *Syphacia muris* (anal tape test), sialodacryoadenitis virus, *Mycoplasma pulmonis*, Sendai virus, pneumonia virus of mice, rat virus, H-1 virus, reovirus 3, rat parvovirus, mouse adenovirus, Theiler's murine encephalomyelitis virus, lymphocytic choriomeningitis virus, Hantaan virus, *Encephalitozoon cuniculi* and cilia-associated respiratory bacillus. Lung sections of sentinels showed no lesions typical of rat respiratory virus infection. Rats were acclimated to the facility for at least 5 d before the start of the study.

Surgery

All surgery was carried out over a period of 3 h. Rats were selected from a rack and sequentially assigned to a treatment group and one surgeon, such that each surgeon operated on at least one rat in each group. Surgeons carried out exploratory laparotomy according to previously published methods in rats that were anesthetized with isoflurane². All surgeons were experienced with the procedure. Each procedure took approximately 10 min. Rats that did not undergo surgery received isoflurane anesthesia for approximately 10 min.

Analgesics

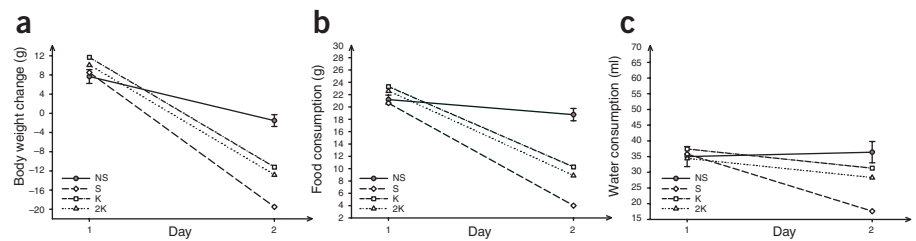
For both experiments we administered preoperative analgesics at the time of anesthetic induction (between 8:30 and 11:30 AM on the day of surgery). For the first experiment we administered postoperative analgesics between 8:00 and 8:30 PM on the day of surgery. Therefore, the dosing interval was approximately 9–12 h. Rats that were injected with saline received 0.04 ml sterile, preservative-free 0.9% saline (Abbott Laboratories, Abbott Park, IL or Hospira, Lake Forest, IL). All injections were intramuscular (i.m.).

We prepared a solution of ketoprofen at the beginning of each experiment by diluting Ketofen (Fort Dodge Animal Health, Fort Dodge, IO) in preservative-free 0.9% saline at the concentrations indicated below. We calculated doses according to rats' average body weight on the day before surgery. For the first experiment, rats that received a dose of 3 mg ketoprofen per kg body weight were given 0.04 ml of a solution with a concentration of 20 mg ketoprofen per ml saline. For the second experiment, rats that received 3 mg ketoprofen per kg body weight were given 0.06 ml of a solution with a concentration of 15 mg ketoprofen per ml saline. Rats that were given 5 mg ketoprofen per kg body weight received 0.11 ml of this same solution.

This method resulted in slight variation from the target dosage. In the first experiment rats received 2.7–2.9 mg ketoprofen per kg body weight. In the

FIGURE 1 | Results of the first experiment (one versus two doses). Measurements for rats immediately before a surgical procedure (day 1) and on the following day (day 2). NS, no surgery and no analgesia; S, surgery and no analgesia; K,

surgery and one dose of ketoprofen (preoperative); 2K, surgery and two doses of ketoprofen (one preoperative and one postoperative). Each dose of ketoprofen was 3 mg per kg body weight. Bars indicate s.e.m. (a) Body weight change. (b) Food consumption. (c) Water consumption. Changes in all three measurements were significantly more pronounced in S, K and 2K groups than in the NS group. Likewise, changes in these measurements were significantly more pronounced in the S group than in the other three groups ($P \leq 0.05$).



second experiment rats that were given the low dose received 2.5–2.7 mg ketoprofen per kg body weight, and rats that were given the high dose received 4.4–5.0 mg ketoprofen per kg body weight.

Necropsy

In the second experiment, after final data collection, all rats that underwent surgery were euthanized by carbon dioxide asphyxiation and cervical dislocation. We carried out a gross necropsy to examine the abdominal and thoracic viscera.

Data analysis

On the day of surgery and on the following day, we calculated daily body weight change and food and water consumption by measuring body weight, food weight and water volume and subtracting the measurements taken on the previous day. We carried out a one-factor analysis of covariance on each parameter using the MIXED procedure in SAS software (version 8.2; SAS Institute, Cary, NC)²⁰. We included changes on the day of surgery as a covariate to adjust for pre-existing differences before treatment. We evaluated changes on the day after surgery by carrying out pairwise *t*-tests on the difference between the means for each pair of groups. We compared six pairs of means in the first experiment and three pairs of means in the second experiment. Therefore, in the first experiment we noted statistical significance for $P \leq 0.008$ (unadjusted) and in the second experiment

for $P \leq 0.017$ (adjusted by the Bonferroni method) to achieve $P \leq 0.05$ (ref. 21). Normality and homogeneity of the results were assessed by Shapiro-Wilk's test and Levene's test, respectively ($P < 0.01$; refs. 22,23).

RESULTS

First experiment (one versus two doses)

Both assumptions of normality and homogeneity were satisfied. One rat in the group that received a single dose of ketoprofen died because of surgery, so we excluded it from the data analysis. Results for body weight change, food consumption and water consumption are plotted in Figure 1.

On the day after surgery, reductions in food and water consumption and body weight were significantly more pronounced in rats that underwent surgery compared with rats that did not. In both groups of rats treated with ketoprofen, changes in food and water consumption and body weight were significantly less pronounced than in rats that underwent surgery and did not receive ketoprofen. There were no significant differences in any of the measured values between rats that received one dose of ketoprofen and rats that received two doses (Table 1).

Second experiment (high versus low doses)

Both assumptions of normality and homogeneity were satisfied. Results for body weight change, food consumption and water consumption are plotted in Figure 2.

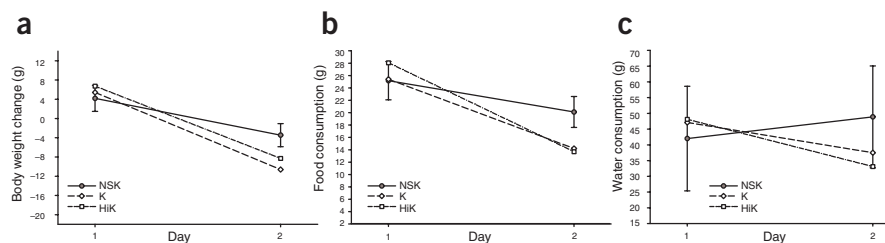
TABLE 1 | Results of the first experiment (one versus two doses): statistically significant differences ($P \leq 0.05$) between groups of rats in measurements taken on the day after surgery

Groups compared	Body weight change (g)	Food consumption (g)	Water consumption (ml)
S vs. NS	-17.99 (-1191%)	-14.76 (-79%)	-18.8 (-52%)
K vs. NS	-9.71 (-643%)	-8.49 (-45%)	—
2K vs. NS	-11.33 (-750%)	-9.89 (-53%)	—
K vs. S	8.28 (42%)	6.27 (157%)	13.7 (78%)
2K vs. S	6.66 (34%)	4.87 (122%)	10.7 (61%)

Values represent the difference between the two groups compared (results for first group – results for second group) in mean measurements of body weight change, food consumption and water consumption taken on the day after surgery (percent change). NS, no surgery and no analgesia; S, surgery and no analgesia; K, surgery and one dose of ketoprofen (preoperative); 2K, surgery and two doses of ketoprofen (one preoperative and one postoperative). Each dose of ketoprofen was 3 mg per kg body weight. There were no significant differences between K and 2K groups.

FIGURE 2 | Results of the second experiment (high versus low doses). Measurements for rats immediately before a surgical procedure (day 1) and on the following day (day 2). NSK, no surgery and 3 mg ketoprofen per kg body weight; K,

surgery and 3 mg ketoprofen per kg body weight (preoperative); HiK, surgery and 5 mg ketoprofen per kg body weight (preoperative). Bars indicate s.e.m. (a) Body weight change. Weight loss in K rats was significantly greater than in NSK rats. (b) Food consumption. In K and HiK rats, food consumption was significantly lower than in NSK rats. (c) Water consumption.



On the day after surgery, reductions in food consumption and body weight were significantly more pronounced in rats that received a low dose of ketoprofen and underwent surgery than in rats that received a low dose of ketoprofen and did not undergo surgery. In rats that received a high dose of ketoprofen, only food consumption was significantly lower than in rats that did not undergo surgery (Table 2).

At necropsy we did not observe important abnormalities in any of the rats.

DISCUSSION

The results of these experiments indicate that a single i.m. preoperative dose of 3 mg ketoprofen per kg body weight is as effective as two doses of 3 mg ketoprofen per kg body weight (preoperative and postoperative) or a single preoperative dose of 5 mg ketoprofen per kg body weight in improving food and water consumption and alleviating body weight loss in Sprague-Dawley rats 24 h after surgery. These results bridge the findings of previous studies^{2,7,18}.

When comparing the effects of one or two daily doses of ketoprofen on food and water consumption and body weight change, we decided to measure these parameters approximately 24 h after surgery rather than 12 h and 24 h. This design permitted us to include

food and water consumption during both phases of the light:dark cycle in a single evaluation. This has previously been shown to be more reliable than measurements that only consider the light phase¹⁷.

It has been suggested to us that it might be unnecessary to evaluate the effect of two daily doses of ketoprofen, as the pharmacokinetic characteristics of this drug indicate that it provides 24 h of analgesic effect in rats. In our review of the literature, however, we did not find consistent data to draw this conclusion. The half-life of ketoprofen in rats ranges between 9.5 and 13 h (refs. 24, 25). In humans the therapeutic range for ketoprofen blood concentration is 0.4–6 µg per ml (ref. 16). Different pharmacokinetic studies in rats have shown variable blood concentrations even when similar dosages of ketoprofen were administered. In some studies ketoprofen concentrations exceeded the human therapeutic range 24 h after dosing, and in other studies concentration never reached that level^{24–27}. Therefore, the pharmacokinetic data were inconclusive, and a model that measured response to therapy was needed. We were not able to find data in the literature on the duration of activity for ketoprofen in rats.

When we compared rats that received 5 mg ketoprofen per kg body weight with rats that received 3 mg per kg body weight, we did not observe significant differences between the two groups in food and water consumption or body weight change. We noted that rats that received the higher dose did not lose a significant amount of weight after surgery, whereas post-surgical weight loss in rats that received the lower dose was significant compared with controls that did not undergo surgery. Food consumption, however, was significantly lower in both groups of rats compared with controls, so the additional benefit was only marginal. Neither dosage was associated with gross lesions of gastrointestinal toxicity, though there may not have been sufficient time for a lesion to develop by the time of necropsy.

In the second experiment, the relative magnitude of effects in the measured parameters was lower than the magnitude seen in the first experiment and in our

TABLE 2 | Results of the second experiment (high versus low doses): statistically significant differences ($P \leq 0.05$) between groups of rats in measurements taken on the day after surgery

Groups compared	Body weight change (g)	Food consumption (g)
NSK vs. K	-7.15 (-207%)	-5.90 (-29%)
NSK vs. HiK	—	-6.42 (-32%)

Values represent the differences between the two groups compared (results for first group – results for second group) in mean measurements of body weight change and food consumption taken on the day after surgery (percent change). There were no statistically significant differences between groups in water consumption. NSK, no surgery and 3 mg ketoprofen per kg body weight (preoperative); K, surgery and 3 mg ketoprofen per kg body weight (preoperative); HiK, surgery and 5 mg ketoprofen per kg body weight (preoperative). There were no statistically significant differences between K and HiK groups.

previous studies, though the model and procedures we used were essentially identical². The reason for this difference is not clear. We noted that in rats in the second experiment that received ketoprofen and did not undergo surgery, reduction of food and water consumption and body weight loss was less pronounced than in previous experiments. Therefore, the difference in magnitude is likely to be related to a variable that was specific to this run of the model. It is also possible that the use of different anesthesia and surgical personnel in previous studies affected the amount of intra-operative stimulation that occurred. Overall, the data from this model were consistent with data from previous experiments in that they enabled differentiation between analgesic-treated and control rats and indicated differences in the degree of postoperative pain that might be expected with different levels of stimulation of pain receptors^{2-4,17}. It is possible that with a higher degree of pain stimulation, the two dosages might have differential effects. In addition, according to the dose-related kinetics of ketoprofen in rats, higher dosages would be expected to provide a longer duration of action if elimination pathways became saturated²⁷. This effect might also be more significant with a greater pain stimulus.

Additional published models for post-procedural pain assessment in rodents have monitored behavioral changes; physiological parameters such as heart rate, activity and blood pressure after surgery; reflexive response from incisional allodynia; and hyperalgesia from induced pancreatitis^{7,8,15,28-30}. These studies did not demonstrate that postoperative pain relief required ketoprofen dosages that were substantially different from the doses we used in our model. We have not seen a direct comparison between the various models to determine their relative sensitivity. It has been shown that traditional analgesiometric assays differ in their ability to compare the effects of analgesics with different mechanisms of action and that this is probably because of the different mechanisms of pain stimulated by the assays³¹. Pain from an abdominal surgical procedure such as that used in this study probably stimulates multiple pain pathways. Models that measure overall response to multiple pain pathways may be equally useful in assessing pain compared with models that evaluate more specific responses and specific pain pathways.

In conclusion, in measures of food and water consumption and body weight in rats that underwent a simple laparotomy, a single preoperative dose of 3 mg ketoprofen per kg body weight provided an analgesic benefit similar to that of two perioperative doses of 3 mg per kg body weight or a single preoperative dose of 5 mg per kg body weight. Future studies may determine whether a different dosage or a longer dosing interval would be more appropriate for different surgical procedures.

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COMPETING INTERESTS STATEMENT

The authors declare no competing financial interests.

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