Assessment of the analgesic effects of ketoprofen in ducks anesthetized with isoflurane

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**Objective**—To determine whether administration of ketoprofen would have analgesic effects in spontaneously breathing ducks anesthetized with isoflurane.

**Animals**—13 healthy adult wild-strain Mallard ducks.

**Procedure**—Each duck was anesthetized twice in a crossover study design with 6 days between randomized treatments. Ducks were given ketoprofen (0.5 to 2.0 mg/kg, IM) or saline (0.9% NaCl) solution after a constant plane of anesthesia was established. Analgesia was assessed by measuring heart and respiratory rates and duration of application of a noxious stimulus. The noxious stimulus was applied 30, 50, and 70 minutes after drug administration and was maintained until gross purposeful movements were seen or for a maximum of 5 seconds.

**Results**—At all 3 evaluation times, heart rate increases in response to the noxious stimulus were greater when ducks were given saline solution than when they were given ketoprofen. The increase in respiratory rate in response to the noxious stimulus was greater when ducks were given saline solution than when they were given ketoprofen only 70 minutes after drug administration. When ducks were given ketoprofen, duration of the noxious stimulus was significantly longer 50 and 70 minutes, but not 30 minutes, after drug administration.

**Conclusions and Clinical Relevance**—Ketoprofen reduced the increase in heart and respiratory rates associated with application of a noxious stimulus in spontaneously breathing adult Mallard ducks anesthetized with isoflurane delivered at approximately 2.9%, suggesting that ketoprofen had analgesic effects in these ducks. The onset of analgesic effects may be longer than 30 minutes in some ducks. (Am J Vet Res 2002;63:821–826)

In mammals, nonsteroidal anti-inflammatory drugs (NSAID) produce analgesia by decreasing inflammation at the site of injury and through CNS effects. 3 Prostaglandins are important local mediators of inflammation and pain, and NSAID control pain in mammals predominantly by inhibiting cyclooxygenase enzymes, preventing production of prostaglandins. Because prostaglandins are involved in the modulation of pain responses in birds and because the physiologic mechanisms involving prostaglandins in birds are similar to those in mammals, it seems reasonable to expect that NSAID would produce analgesia in birds.

Evidence of the analgesic effects of NSAID in birds, however, is contradictory. In 1 study, topical application of phenylbutazone to the beaks of chickens resulted in maintenance of feed intake during the first 24 hours after bead trimming, compared with a decrease in intake among chickens that were not treated. In a separate study, lame chickens preferentially selected food containing carprofen, compared with untreated food, and in another study, carprofen increased the speed and walking ability of rapidly growing broiler chickens with chronic lameness. However, in a study of Mallard ducks undergoing a skin incision, ketoprofen (0.5 to 2.0 mg/kg, IM) did not provide any detectable analgesia.

The purpose of the study reported here was to determine whether administration of ketoprofen at a higher dose (5 mg/kg, IM) would have analgesic effects in isoflurane-anesthetized Mallard ducks. We elected to study this dose because pharmacologic effects of ketoprofen have been demonstrated after IM administration at a dose of 3 mg/kg in Mallard ducks.

One method of evaluating analgesic effects of a drug is to measure responses to a noxious stimulus. However, stress responses may influence responses to noxious stimuli, and separation from social companions can alter pain perception in gregarious species. For example, birds held and tested in familiar pens are significantly less able to cope with noxious stimuli than are birds tested in novel pens. In addition, repeated exposure to noxious stimuli can induce tonic immobility in birds, which can reduce responsiveness for several seconds to several hours. Mallard ducks available for use in this study were a wild strain and had not been habituated to handling. Thus, ducks were anesthetized with isoflurane during the study to reduce stress associated with handling, separation from the group, and manipulation and to allow for an uncomplicated assessment of responses to a noxious stimulus.

The potency of analgesic drugs is often determined by assessing the drug’s ability to reduce the minimum alveolar concentration (MAC) of an inhalant anesthetic. In mammals and birds, for instance, studies have shown that opioids reduce the requirement for inhalant anesthetics during anesthesia. However, NSAID have not been shown to reliably reduce the MAC of inhalant anesthetics in mammals or birds, therefore, we did not evaluate the effects of ketoprofen on the MAC of isoflurane in these birds.
Changes in heart and respiratory rate can be used as indicators of acute pain and were evaluated in the present study. Although changes in blood pressure are also indicative of acute pain, direct measurement of blood pressure in birds necessitates an incision for placement of an arterial catheter. Trauma and inflammation associated with such an incision would likely result in nociceptive information reaching the spinal cord, causing central sensitization and prolonged changes in CNS function that might influence responses to subsequent noxious stimuli. Therefore, we elected to not measure blood pressure in these birds.

Similarly, trauma and inflammation associated with a noxious stimulus could themselves result in central sensitization and alterations in responses to subsequent stimuli. In addition, NSAID can accumulate in areas of inflammation, which would also alter responses to subsequent stimuli. In the present study, the noxious stimulus that was used was application of a hemostat to the leg. To limit traumatic damage associated with application of the hemostat, the ends of the hemostat were covered with plastic tubing and bent to prevent complete closure. In addition, the hemostat was applied for a maximum of 5 seconds. This was in accordance with a previous study of analgesia in birds, in which a potentially damaging noxious stimulus was applied for a limited time.

**Materials and Methods**

**Ducks**—Thirteen healthy adult female Mallard ducks (Anas platyrhynchos) were used in the study. Ducks were the F2 progeny of wild adults. Mean ± SD weight was 1.35 ± 0.18 kg. Ducks were maintained and treated in accordance with guidelines of the Canadian Council on Animal Care as defined by the Guide to the Care and Use of Experimental Animals. The study protocol was approved by the University of Saskatchewan Animal Care Committee.

**Experimental protocol**—Each duck was anesthetized twice in a crossover study design. The order of treatments was randomized, with a minimum of 6 days between treatments. For each treatment, anesthesia was induced with 5% isoflurane in oxygen delivered through a nonrebreathing coaxial system. Oxygen flow was set at 1 L/min. Ducks were maintained in sternal recumbency on a warm-water heating pad and were covered with a towel to maintain body temperature between 38 and 39 C.

Isoflurane concentration was altered in each duck to maintain a similar plane of anesthesia. Therefore, the isoflurane concentration was adjusted as necessary to maintain spontaneous respiration, a grade-2 response of the nictitating membrane to opening and closing of the eyelids, and a lack of response to wing and leg extension. Once ducks had reached an acceptable plane of anesthesia, ketoprofen (5 mg/kg) or an equal volume of saline (0.9% NaCl) solution was given IM in the pectoral muscles.

**Data collection**—Ducks were maintained at a constant plane of anesthesia for 30 minutes prior to administration of ketoprofen or saline solution. Before each application of the noxious stimulus, heart and respiratory rates were recorded for 1 minute by continuous recording. A noxious stimulus was then applied, and changes in heart and respiratory rates were recorded by continuous recording for at least 1 minute, along with duration of stimulus application. Ducks were returned to the original plane of anesthesia, and 20 minutes after application of the first noxious stimulus (50 minutes after treatment), a second noxious stimulus was applied. Changes in heart and respiratory rates and duration of stimulus application were again recorded, and ducks were again returned to the original plane of anesthesia. Twenty minutes after the second noxious stimulus was applied (70 minutes after treatment), a third and final noxious stimulus was applied. Changes in heart and respiratory rates and duration of stimulus application were recorded a final time, and ducks were allowed to recover from anesthesia.

Animals. The study protocol was approved by the University and left inguinal region, and the electrocardiogram was monitored continuously. An elbow adapter was positioned between the endotracheal tube and the anesthesia circuit, and a thermocouple was passed through an opening in this adapter to allow monitoring of ventilation.

Immediately after endotracheal intubation, platinum electrodes were placed subcutaneously in the left and right patagium and left inguinal region, and the electrocardiogram was monitored continuously. An elbow adapter was positioned between the endotracheal tube and the anesthesia circuit, and a thermocouple was passed through an opening in this adapter to allow monitoring of ventilation.

End-tidal isoflurane concentration was measured at the time of application of the noxious stimulus with a polyethylene catheter inserted through the opening in the elbow adapter of the anesthetic circuit; the catheter was connected to an anesthetic gas monitor. The anesthetic gas monitor was
calibrated with room air and 1.5% isoflurane prior to the start of each experiment. Aspiration rate of the anesthetic gas monitor was set at 50 ml/min.

A single investigator who was unaware of whether ducks had been given ketoprofen or saline solution delivered the noxious stimulus and recorded and evaluated all data.

Statistical analyses—Data are reported as mean ± SD. For each duck, change in heart rate 0, 25, and 45 seconds after each application of the noxious stimulus (30, 50, and 70 minutes after administration of ketoprofen or saline solution) was calculated by subtracting the baseline value from the value obtained 0, 25, and 45 seconds after application of the stimulus. Change in respiratory rate after each application of the noxious stimulus was calculated by subtracting respiratory rate before application of the stimulus from respiratory rate after application. A Kolmogorov-Smirnov test was used to determine whether values were normally distributed. Changes in heart and respiratory rates and duration of noxious stimulus application were compared among treatments and times after injection by use of general linear model analysis and repeated-measures ANOVA. A sign test was used to determine whether differences existed in the direction of change in heart rate and the graded response to the noxious stimulus among treatments. Commercial software was used for all analyses; values of \( P \leq 0.05 \) were considered significant.

Results

Fifty minutes after drug administration, 11 of the 13 ducks had higher heart rates immediately after application of the noxious stimulus (0 seconds) when given saline solution than when given ketoprofen; this proportion was significantly (sign test; \( P = 0.011 \)) higher than 50%, the proportion expected on the basis of chance alone. However, at 30 and 70 minutes, the proportions of ducks that had higher heart rates immediately after application of the noxious stimulus when given saline solution than when given ketoprofen (9/13) were not significantly (sign test; \( P = 0.113 \)) different from 50%. For all 3 applications of the noxious stimulus (30, 50, and 70 minutes), mean change in heart rate immediately after application of the noxious stimulus (0 seconds) was significantly (\( P = 0.01 \)) greater when ducks were given saline solution than when they were given ketoprofen (Fig 1). At 50 and 70 minutes, but not at 30 minutes, change in heart rate 25 and 45 seconds after application of the noxious stimulus was significantly (\( P = 0.002 \) and 0.013, respectively) different when ducks were given saline solution than when they were given ketoprofen. However, at all 3 times, a significant effect of time on change in heart rate was detected.

End-tidal isoflurane concentration was not significantly different between treatments. End-tidal isoflurane concentrations 30, 50, and 70 minutes after administration of ketoprofen were 2.9 ± 0.6, 2.9 ± 0.5,
The proportion of ducks with higher heart rates immediately after application of the noxious stimulus (0 seconds) when given saline solution, versus ketoprofen, was lower 70 minutes after drug administration than it had been 50 minutes after drug administration.

(Fig 3). When response to the noxious stimulus was graded, the proportion of ducks that had a higher grade when saline solution was given than when ketoprofen was given was significantly different from 50% at 50 and 70 minutes ($P = 0.008$ and 0.035, respectively), but not at 30 minutes ($P = 0.113$).

Redness and indentation of the skin overlying the midpoint of the metatarsal bone were noticeable after the first application of the noxious stimulus. Skin was damaged after the third application of the noxious stimulus, and scarring was evident 6 days later.

**Discussion**

In the present study, administration of ketoprofen reduced the increases in heart and respiratory rates associated with application of a noxious stimulus in spontaneously breathing adult female Mallard ducks anesthetized with isoflurane delivered at approximately 2.9%. In addition, mean time the noxious stimulus could be applied before gross purposeful movements were seen (up to a maximum of 5 seconds) was significantly longer when ducks were given ketoprofen than when they were given saline solution. We conclude, therefore, that administration of ketoprofen at a dose of 5 mg/kg to Mallard ducks results in clinically detectable analgesia. However, use of clinical signs to determine plane of anesthesia in the present study and hypercarbia associated with administration of high concentrations of isoflurane may have confounded our results. Thus, additional research may be needed to verify the efficacy of ketoprofen in birds.

In the present study, ketoprofen's effects on heart rate were less evident 30 minutes after administration, compared with effects detected 50 and 70 minutes after drug administration. Potentially, this may have been attributable to the low number of ducks in the study, which reduced the power to detect differences. Alternatively, the onset of analgesia may have been longer than 30 minutes in some ducks because of individual variations in uptake and distribution of the drug. In a previous study, administration of ketoprofen (5 mg/kg) resulted in $> 82\%$ suppression in thromboxane B2 activity by 15 minutes, indicating that absorption was rapid. However, degree of analgesia does not necessarily correlate with degree of suppression of thromboxane B2 activity, as NSAID exert their analgesic effects through a variety of peripheral and central mechanisms. Analgesia associated with mechanisms of action of NSAID not related to local prostaglandin synthesis may take longer to become fully active. Proposed mechanisms of action of NSAID unrelated to prostaglandin synthesis include alterations in spinal nociceptive processing through cellular or intracellular mechanisms possibly involving interference with G-protein-mediated signal transduction, central activation of endogenous opioid peptides, blockade of the release of serotonin, and inhibition of excitatory amino acids involved in N-methyl-D-aspartate receptor activation.

The proportion of ducks with higher heart rates immediately after application of the noxious stimulus (0 seconds) when given saline solution, versus ketoprofen, was lower 70 minutes after drug administration than it had been 50 minutes after drug administration.
The power to detect significant differences may have been low because of the small sample size. However, isoflurane induces dose-dependent respiratory depression, and as ventilation was not controlled in this study, it is possible that PaCO₂ increased over time. In the present study, end-tidal isoflurane concentration was approximately 2.9%, and in a previous study, spontaneously breathing Sandhill cranes (Grus canadensis) anesthetized with isoflurane at 2 times the MAC in this species (approx 2.8% isoflurane) had PaCO₂ values > 100 mm Hg. Hypercarbia has been associated with increases in vagal tone and bradycardia and may induce narcosis, which may have lessened differences in the change in heart rate in response to the noxious stimulus. However, end-tidal isoflurane concentration did not differ when ketoprofen was given versus when saline solution was given. Therefore, the effects of hypercarbia on heart and respiratory rates and on responses to the noxious stimulus would have been the same for each treatment. Accordingly, we believe that significant differences between treatments represent a true analgesic effect of ketoprofen. However, these results should be confirmed with more controlled and defined conditions.

In the present study, a significant difference between treatments in regard to change in respiratory rate was detected only 70 minutes after drug administration, and not at 30 or 50 minutes. This suggests that under the conditions of the present study, respiratory rate may not have been as good an indicator of analgesia as heart rate. In particular, hypercarbia may have blunted changes in respiratory frequency and volume associated with increases in vagal tone and bradycardia and may induce narcosis, which may have lessened differences in the change in heart rate in response to the noxious stimulus. Therefore, the effects of hypercarbia on heart and respiratory rates and on responses to the noxious stimulus would have been the same for each treatment. Accordingly, we believe that significant differences between treatments represent a true analgesic effect of ketoprofen. However, these results should be confirmed with more controlled and defined conditions.

Although we attempted to reduce traumatic damage associated with the noxious stimulus by altering the hemostats and minimizing the duration of application, some tissue damage was evident. It is possible that tissue injury and inflammation could have made the ducks more sensitive to the second and third applications of the noxious stimulus (ie, at 50 and 70 minutes), as tissue injury and inflammation can affect the peripheral and central nervous systems in mammals and alter sensitivity to subsequent stimuli. This sensitization may be characterized by a lower threshold of activation, an increased response to noxious stimuli, a shorter response latency, a longer duration of response to stimulation, an increased response to a given stimulus intensity or spontaneous activity, and a spread of pain and hyperalgesia to uninjured tissue. However, duration of application of the stimulus was significantly longer when ducks were given ketoprofen than when they were given saline solution, yet fewer changes in heart and respiratory rates were seen when ducks were given ketoprofen. This suggests that ketoprofen produced analgesia even if there was increased sensitivity 50 and 70 minutes after drug application.

References