Effect of meloxicam and butorphanol on minimum alveolar concentration of isoflurane in rabbits

Patricia V. Turner, DVM, DVSc; Carolyn L. Kerr, DVM, PhD; Amanda J. Healy, BSc; W. Michael Taylor, DVM

Objective—To determine the effects of meloxicam and butorphanol on minimum alveolar concentration of isoflurane (MAC\textsubscript{ISO}) in rabbits.

Animals—10 healthy young adult female rabbits.

Procedure—Rabbits were anesthetized with isoflurane on 3 occasions in a blinded, randomized complete block design to determine the MAC\textsubscript{ISO} associated with administration of meloxicam (0.3, 0.4, or 1.5 mg/kg, PO) and butorphanol (0.4 mg/kg, IV). The MAC\textsubscript{ISO} was determined by use of a paw clamp technique as the end-tidal concentration of isoflurane halfway between the values that allowed or inhibited purposeful movement. Rectal temperature, end-tidal CO\textsubscript{2} concentration, heart rate, oxygen saturation, and arterial blood pressure were measured to evaluate cardiopulmonary function.

Results—Mean ± SE MAC\textsubscript{ISO} in saline (0.9% NaCl) solution–treated rabbits was 2.49 ± 0.07% and was not significantly different from that associated with administration of meloxicam at 0.3 mg/kg (2.66 ± 0.07%) or 1.5 mg/kg (2.66 ± 0.07%). Butorphanol significantly reduced the MAC\textsubscript{ISO} to 2.30 ± 0.07% when administered with saline solution alone, 2.27 ± 0.07% when administered with 0.3 mg of meloxicam/kg, and 2.33 ± 0.07% when administered with 1.5 mg of meloxicam/kg. The percentage reduction in MAC\textsubscript{ISO} was significantly greater for rabbits that received butorphanol and meloxicam at either dose, compared with butorphanol and saline solution.

Conclusions and Clinical Relevance—Results indicated that meloxicam does not have a direct isoflurane-sparing effect and does not interfere with the anesthetic-sparing effect of butorphanol in rabbits. (Am J Vet Res 2006;67:770–774)

Meloxicam is a novel COX-2 selective NSAID that has been used extensively as an analgesic agent in humans and, more recently, in some companion animals. Unlike many other NSAIDs, meloxicam retains high bioavailability after oral administration and has a relatively long half-life, making it an attractive analgesic for use in veterinary practice. It is used empirically as an analgesic in rabbits because there are no published pharmacokinetic or clinical data for its use in this species.

Nonsteroidal anti-inflammatory drugs are routinely used in companion animals to provide analgesia and may be combined with opioids to provide synergistically increased analgesic potency. The combination of an NSAID and an opioid such as butorphanol, a partial opioid agonist-antagonist, is commonly used with an inhalant agent such as isoflurane to optimize intra- and postoperative analgesia and reduce inflammation. For example, preemptive administration of butorphanol (0.2 mg/kg, IV) and meloxicam (0.2 mg/kg, IV) to dogs undergoing stifle joint surgery empirically provides superior postoperative analgesia, compared with 2 doses of butorphanol alone. In addition to providing more efficacious analgesia, use of these agents in combination allows all drugs to be used at doses less than those used when a single drug is administered, minimizing the potential for adverse effects for any 1 drug.

The potency of an inhalant anesthetic agent can be determined objectively by measurement of the amount of anesthetic required to prevent movement in 50% of individuals in response to a noxious stimulus and is termed MAC. This standard technique can also be used to study the relative analgesic effects of various drugs on anesthetic requirements. The MAC\textsubscript{ISO} in rabbits is 2.08% as measured by use of the paw-clamp technique. Butorphanol is commonly added to the anesthetic regimen of rabbits to provide intraoperative analgesia; however, butorphanol-sparing effects on MAC\textsubscript{ISO} in rabbits have not been reported. In cats, butorphanol given at either 0.08 or 0.8 mg/kg, IV, induces an 18% to 19% reduction in MAC\textsubscript{ISO}. This is somewhat less than the maximal reduction in MAC\textsubscript{ISO} of 28% induced in cats by treatment with morphine at 1 mg/kg, IV, and the difference was attributed to the partial opioid agonist-antagonist activity of butorphanol, compared with the pure μ-agonist activity of morphine. Whether a COX-2 preferential inhibitor, such as meloxicam, can potentiate the anesthetic-sparing effects of butorphanol on MAC\textsubscript{ISO} is important information for providing optimal intraoperative anesthesia and analgesia for rabbits and other species, including humans. Determining whether meloxicam and butorphanol can reduce MAC\textsubscript{ISO} has other important implications for all species because decreasing the amount of inhalant anesthetic agent required for patients decreases dose-related adverse effects of car-
diuresis, respiratory suppression, anesthetic costs, and pollution from waste gases.

The purpose of the study reported here was to determine the effects of meloxicam and butorphanol on MAC\textsubscript{ISO} in rabbits.

**Materials and Methods**

**Rabbits**—Ten 3-month-old female New Zealand White rabbits\textsuperscript{a} that weighed approximately 3 kg were group housed in floor pens on kiln-dried pine shavings on a 12:12-hour light-dark cycle at 20 ± 4°C and relative humidity of 30% to 70%. Rabbits were fed twice daily and provided with fresh water ad libitum. Vendor surveillance reports indicated that rabbits were free of known bacterial, viral, and parasitic pathogens. Rabbits were acclimated extensively prior to study initiation. The facilities and procedures were in compliance with the Animals for Research Act of Ontario and the Guidelines of the Canadian Council on Animal Care. The University of Guelph Animal Care and Use Committee approved the study protocol.

**Experimental design**—The study design was a complete-block crossover design randomized on rabbit and treatment order to account for period and carryover effects. The investigators were unaware of treatments administered to each rabbit prior to induction of anesthesia. Each rabbit was anesthetized 3 times, and each period of anesthesia was preceded by 1 of 3 oral administrations (meloxicam,\textsuperscript{b} 0.3 mg/kg; meloxicam, 1.5 mg/kg; or an equivalent volume of saline [0.9% NaCl] solution). After initial MAC\textsubscript{ISO} determination, butorphanol tartrate (0.4 mg/kg, IV) was administered, and MAC\textsubscript{ISO} was measured again after a 30-minute equilibration period. A 14-day washout period was used between subsequent anesthetic periods. Rabbits were euthanatized at completion of the third trial.

**Anesthesia**—Each rabbit received the assigned oral administration 1 hour prior to anesthetic induction with isoflurane\textsuperscript{c} in oxygen by facemask. The trachea was intubated, and anesthesia was maintained by use of a Bain breathing circuit at an oxygen flow rate of 2 L/min. During a 30-minute anesthetic equilibration period in which rabbits were maintained at a stable end-tidal isoflurane concentration, rabbits were instrumented with a rectal temperature probe,\textsuperscript{d} an arterial blood oxygen saturation sensor,\textsuperscript{d} and a direct arterial blood pressure catheter in the auricular artery.\textsuperscript{e} Intratracheal end-tidal concentrations of isoflurane and CO\textsubscript{2} were monitored by use of an infrared absorption spectrophotometer\textsuperscript{f} that was calibrated prior to the start of each experiment with a standardized calibration gas mixture designed for the analyzer.\textsuperscript{f} Rabbits were mechanically ventilated, and end-tidal CO\textsubscript{2} concentration was maintained from 30 to 40 mm Hg. Rectal temperature was maintained at normothermia by use of a circulating water blanket. A catheter was inserted into the auricular vein, and lactated Ringer’s solution was administered at a rate of 10 mL/kg/h. Just prior to each MAC\textsubscript{ISO} determination, arterial blood pressure\textsuperscript{e} and heart rate were recorded.

The MAC\textsubscript{ISO} value was determined according to established techniques in rabbits by use of a paw clamp as the nociceptive stimulus and a bracketing technique for verifying MAC\textsubscript{ISO}.\textsuperscript{g} Briefly, after the anesthesia equilibration period, a paw of the hind limb was clamped with sponge forceps, 24 cm in length, with protective plastic tubing on each jaw and held until gross purposeful movement occurred or 15 seconds had elapsed. If no response occurred, end-tidal isoflurane concentration was decreased by 0.1%, the anesthetic plane was stabilized for at least 20 minutes, and the MAC\textsubscript{ISO} was retested. If the response was positive, the end-tidal isoflurane concentration was increased by 0.1%, the anesthetic plane was stabilized for at least 20 minutes, and the MAC\textsubscript{ISO} was retested. The MAC\textsubscript{ISO} was taken as the value midway between the highest value at which purposeful movement was detected and the lowest value that prevented purposeful movement and was determined in duplicate.

**Statistical analysis**—All results are expressed as mean ± SE. Percentage reduction of baseline MAC\textsubscript{ISO} after treatment with butorphanol was calculated as follows: (MAC\textsubscript{ISO} baseline – MAC\textsubscript{ISO} + butorphanol)/MAC\textsubscript{ISO} baseline X 100. Statistical analysis was performed by use of a general linear mixed model with rabbit and treatment as whole-plot variables and pre- and posttreatment with butorphanol as split-plot variables, respectively.\textsuperscript{h} A Shapiro-Wilk test was conducted on the residuals to confirm normal distribution of the data. Significance was set at a value of P < 0.05.

**Results**

Mean MAC\textsubscript{ISO} in saline solution–treated rabbits was 2.49 ± 0.07%. Treatment with meloxicam alone at either 0.3 or 1.5 mg/kg, PO, resulted in MAC\textsubscript{ISO} of 2.56 ± 0.07% and 2.66 ± 0.07%, respectively; however, these values were not significantly different from the MAC\textsubscript{ISO} value determined for saline solution alone (P = 0.38 and 0.06, respectively).

Addition of butorphanol (0.4 mg/kg, IV) resulted in a consistent and significant (P = 0.002) reduction in MAC\textsubscript{ISO} from baseline (saline solution alone) for all 3 groups (Figure 1). The absolute mean MAC\textsubscript{ISO} values determined after administration of butorphanol were not significantly different for all groups (approx 2.5 ± 0.07%). The absolute reductions from baseline associated with administration of butorphanol and meloxicam at 0.3 and 1.5 mg/kg were 0.29% and 0.33%, respectively, and were significantly different from the 0.19% reduction detected with butorphanol in saline solution–treated rabbits (P = 0.005 and P < 0.001, respectively); differences between the 2 meloxicam groups were not significant (P = 0.271). Addition of butorphanol induced percentage reductions from values obtained after administration of saline solution or both doses of meloxicam alone of 7.63%, 13.33%, and 12.41%, respectively. Mean ± SE time required for

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**Figure 1**—Mean ± SE MAC\textsubscript{ISO} after administration of saline (0.9% NaCl) solution (SAL), meloxicam (MXC; 0.3 or 1.5 mg/kg), and butorphanol (BUP) in 10 rabbits. *Significant (P < 0.05) difference from value in saline solution group. $\dagger$Significant (P < 0.05) difference from the saline and meloxicam pre-treatment groups, following addition of butorphanol.
MAC determinations after the initial equilibration period was 82 ± 5 minutes.

Administration of meloxicam led to nonsignificant, dose-dependent decreases in heart rate and increases in blood pressure at MACISO (Table 1). Butorphanol injection resulted in further decreases in heart rate at the time of MACISO determination in rabbits pretreated with saline solution or meloxicam at 0.3 mg/kg and decreases in blood pressure of all rabbits, regardless of pretreatment.

### Discussion

Use of MAC values to study potential analgesic interactions between an NSAID and an opioid provides a relevant objective assessment of analgesic efficacy and minimizes subjective error that may occur when assessing postoperative pain scores in animals. In rabbits, meloxicam given PO alone at either 0.3 or 1.5 mg/kg had no effect on MACISO. Despite this, the values obtained for MACISO associated with either dose of meloxicam plus butorphanol were almost identical to those obtained with saline solution and butorphanol alone (approx 2.30%), and this indicated that there was no disadvantage in terms of inhalant requirements in giving these 2 drugs together for perioperative analgesic management of rabbits. Significant dose-related differences were detected in the magnitude of the relative reductions of MACISO obtained when rabbits had been pretreated with meloxicam. With saline solution alone, butorphanol induced a percentage reduction of MACISO of 7.63%, whereas pretreatment with meloxicam at 0.3 or 1.5 mg/kg induced significantly greater reductions of 11.33% and 12.41%, respectively. Because meloxicam did not have any effect on its own, this may be suggestive of a beneficial pharmacologic interaction between meloxicam and butorphanol.

A recent study of the effects of meloxicam on reduction of MACISO by morphine in outbred rats determined that meloxicam did not potentiate the anesthetic-sparing effects of morphine. In the rat study, there were no differences in the percentage reductions in MACISO associated with administration of meloxicam plus morphine versus morphine alone. The reasons for these differences are unclear but may be because of species-specific variations in drug response; sex or strain-related differences in metabolism of meloxicam or MACISO; analgesic potency of morphine, compared with butorphanol; or issues related to study design. In the rat study reported by Santos et al., study design issues may have reduced statistical power and influenced results because different groups of rats were evaluated for each drug treatment. Morphine induces a profound reduction in MACISO compared with butorphanol, which may obscure more subtle pharmacologic interactions, as has been reported in cats. A study of the effect of another NSAID, flunixin meglumine, on morphine-induced reductions in MACISO in goats also did not find any additional effect when flunixin meglumine was given, potentially because of the 30% reduction in MACISO obtained with morphine alone. Among animals of a species, there may be as much as 20% variation in MAC values but variation is generally <10% in an individual animal. Variation in MAC values may be controlled to some extent by conducting repeated measures within the same animals, as in our study.

Others have reported additive or synergistic effects on reduction of MACISO when NSAIDs have been combined with opioids. A mild additive reduction of MACISO by carprofen and butorphanol has been reported in dogs, and a synergistic reduction was detected with aspirin and morphine in rats. In the dog study, carprofen induced a direct mild isoflurane-sparing effect. The percentage reduction in MACISO values from untreated controls was 6.4% with 2.2 mg of carprofen/kg, 20.3% with 0.4 mg of butorphanol/kg IV, and 29.5% with both carprofen and butorphanol. In the rat study, the effects of aspirin on aminophylline-induced reduction of MACISO were synergistic and similar to the present study; aspirin did not alter MACISO when used alone. Morphine alone (1 mg/kg) induced a 17% reduction in MACISO, and the addition of 30 mg of aspirin/kg resulted in a 32% reduction. The pharmacologic features of these 2 NSAIDs are quite different from meloxicam because carprofen yields equipotent inhibition of COX-1 and COX-2 in vitro, whereas aspirin primarily inhibits COX-1 isozymes.

Mean MACISO value for saline solution–treated rabbits in our study was approximately 20% higher than that reported for rabbits by use of a similar supramaximal stimulus (paw-clamp [2.08%] or tail-clamp technique [2.07%]). This is within the expected variation for a given species and may be related to variations in sex, age, source, and health status of the rabbits used in the different studies. In the present study, all anesthetic procedures were carried out at the same time of day to minimize circadian rhythm effects; however, this information is rarely reported and may also affect response to anesthesia.

The isoflurane-sparing effect of butorphanol has not been reported previously in rabbits. The effect was small but significant and represented only a 7.5% reduction in MACISO from baseline (saline solution treatment only). Marked species differences have been reported for opiate-induced reductions of inhalant anesthetic requirements. Intravenous administration of morphine (2 mg/kg) induces mean MACISO reductions from controls of 35% in rhesus monkeys, 50% in dogs, and 13% in swine, whereas administration of butorphanol (0.05 mg/kg, IV) does not have any effect on MAC of halothane in ponies. In dogs, administration of butorphanol (0.4 mg/kg, IV) results in a 20% reduction in isoflurane requirements, whereas butor-
rabbits at MACISO have been reported to be similarly low. Because of this, use of meloxicam alone before halothane-anesthetized dogs induces transient decreases in arterial pressure, which is not thought to be clinically important. Similarly, butorphanol administration to rabbits was cotreated with meloxicam and butorphanol at 0.3 mg/kg. Meloxicam pretreatment does not interfere with the 2 drugs, which have dissimilar mechanisms of action, to optimize analgesic effects while minimizing the potential for important adverse effects. Results of this study indicated that meloxicam administration alone does not reduce MAC$_{C_{ISO}}$ of rabbits at 0.3 mg/kg. Meloxicam pretreatment does not interfere with the anesthetic-sparing effect of butorphanol in rabbits and has minimal effects on cardiorespiratory variables. Because of this, use of meloxicam alone before surgery is unlikely to induce a clinically important reduction in MAC$_{C_{ISO}}$. If the time to peak plasma concentration of meloxicam in rabbits after oral administration is as long as for other species such as dogs, there may be an advantage to giving the drug before surgery to ensure appropriate postoperative analgesic blood concentration of the drug. Further research is required to determine the pharmacokinetics of meloxicam in rabbits.

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