Effect of intravenous administration of tramadol hydrochloride on the minimum alveolar concentration of isoflurane in rabbits

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Objective—To evaluate the effect of IV administration of tramadol hydrochloride on the minimum alveolar concentration of isoflurane (ISOMAC) that prevented purposeful movement of rabbits in response to a noxious stimulus.

Animals—Six 6- to 12-month-old female New Zealand White rabbits.

Procedures—Anesthesia was induced and maintained with isoflurane in oxygen. A baseline ISOMAC was determined by clamping a pedal digit with sponge forceps until gross purposeful movement was detected or a period of 60 seconds elapsed. Subsequently, tramadol (4.4 mg/kg) was administered IV and the posttreatment ISOMAC (ISOMAC T) was measured.

Results—Mean ± SD ISOMAC and ISOMAC T values were 2.33 ± 0.13% and 2.12 ± 0.17%, respectively. The ISOMAC value decreased by 9 ± 4% after tramadol was administered. Plasma tramadol and its major metabolite (M1) concentrations at the time of ISOMAC T determination varied widely (ranges, 181 to 636 ng/mL and 32 to 61 ng/mL, respectively). Intervals to determination of ISOMAC, and plasma tramadol and M1 concentrations were not correlated with percentage change in the ISOMAC. Heart rate decreased significantly immediately after tramadol administration but by 10 minutes afterward was not different from the pretreatment value. Systolic arterial blood pressure decreased to approximately 60 mm Hg for approximately 5 minutes in 3 rabbits after tramadol administration. No adverse effects were detected.

Conclusions and Clinical Relevance—As administered, tramadol had a significant but clinically unimportant effect on the ISOMAC in rabbits. Higher doses of tramadol may provide clinically important reductions but may result in a greater degree of cardiovascular depression. (Am J Vet Res 2009;70:945–949)

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Abbreviations

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<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>ETISO</td>
<td>End-tidal concentration of isoflurane</td>
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<tr>
<td>HPLC</td>
<td>High-performance liquid chromatography</td>
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<tr>
<td>ISOMAC</td>
<td>Minimum alveolar concentration of isoflurane</td>
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<tr>
<td>ISOMAC T</td>
<td>Minimum alveolar concentration of isoflurane after administration of tramadol hydrochloride</td>
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<tr>
<td>MAC</td>
<td>Minimum alveolar concentration of isoflurane after administration of tramadol hydrochloride</td>
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<tr>
<td>P\textsubscript{\text{ETCO}}\textsubscript{2}</td>
<td>End-tidal partial pressure of carbon dioxide</td>
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<td>SAP</td>
<td>Systolic arterial blood pressure</td>
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Tramadol hydrochloride is an analgesic that has become popular in veterinary medicine, although it has been licensed for use in humans in the United States since 1994.\textsuperscript{1,2} The drug is inexpensive, has a low potential for abuse, and is not controlled by the Drug Enforcement Administration, making it appealing for use in animals; however, only the oral formulation is available in the United States.

The analgesic effectiveness of tramadol results from a complex interaction between opiate, \(\alpha\)-adrenergic, and serotonergic receptor systems.\textsuperscript{1} Tramadol provides analgesia by increasing release and decreasing reuptake of serotonin and norepinephrine in the spinal cord.\textsuperscript{1,3} The parent drug and one of its metabolites, M1 (O-desmethyltramadol), have opioid \(\mu\)-receptor agonist effects,\textsuperscript{1} although the importance of these effects may vary among animal species. When tramadol is administered to humans, there is a lower incidence of adverse effects such as respiratory depression or constipation, compared with the incidence of adverse effects with other \(\mu\)-receptor agonist opioids.\textsuperscript{1,2,3}

Tramadol is reportedly an effective postoperative analgesic for abdominal and orthopedic surgery in dogs and intercostal thoracotomy in cats.\textsuperscript{6–9} In horses, epidural administration of tramadol provides long-term analgesia with no adverse effects.\textsuperscript{10} Results of several studies\textsuperscript{11–13} in rats suggest the drug is also an effective analgesic in that species.
The MAC is defined as the anesthetic concentration at which 50% of anesthetized subjects will respond with gross purposeful movement to a noxious stimulus. It is a measure of the potency of a volatile anesthetic and can be used to compare the efficacy of analgesic and anesthetic drugs.\textsuperscript{14,15} In dogs\textsuperscript{16} and rats,\textsuperscript{17} administration of tramadol significantly reduces the MAC of isoflurane. Whereas SC administration of tramadol does not appear to increase the pressure and thermal thresholds in awake cats,\textsuperscript{18} oral administration reduces the MAC of sevoflurane in the same species.\textsuperscript{19}

To the authors’ knowledge, the analgesic or MAC-reducing effects of tramadol in rabbits have not been reported. The purpose of the study reported here was to evaluate the effects of IV administration of tramadol on the ISOMAC in rabbits. Specifically, it was hypothesized that administration of tramadol would decrease the ISOMAC.

**Materials and Methods**

**Animals**—Six female New Zealand White rabbits (*Oryctolagus cuniculus*), aged 6 to 12 months and weighing 4.0 to 4.6 kg, were included in the study. Rabbits were judged to be healthy on the basis of medical history, results of physical examination and plasma biochemical analysis, serum total protein concentration, and Hct. Rabbits were housed together in pens in a temperature-controlled environment (20°C) with managed lighting (12 hours light and 12 hours dark). All were fed a pelleted diet and water was unrestricted at all times, food was withheld for 12 hours prior to anesthesia. The study protocol was approved by the University of Tennessee Institutional Animal Care and Use Committee.

**Anesthesia and monitoring**—Anesthesia was induced with 4% isoflurane in 100% oxygen (2 L/min), delivered via mask from a pediatric circle anesthetic system. After rabbits were endotracheally intubated with a cuffed 4-mm endotracheal tube, anesthesia was maintained with isoflurane in 100% oxygen (2 L/min) by use of a small animal anesthesia machine.\textsuperscript{2} Rabbits were positioned in right lateral recumbency and ventilated to prevent hypercarbia. The ETISO and P\textsubscript{ETCO\textsubscript{2}}, were monitored continually with an infrared sidestream gas analyzer.\textsuperscript{3} Gas samples were collected from the Y-piece at a flow rate of 50 mL/min. A 24-standard wire gauge (1.5-cm) catheter was placed in a cephalic or saphenous vein for infusion of a balanced, isotonic crystalloid solution\textsuperscript{4} (3 mL/kg/h). Body temperature was measured with an esophageal thermometer;\textsuperscript{5} and a circulating water heating blanket was used to maintain esophageal temperature within the reference range (37.0°C to 39.0°C). Systolic arterial pressure was measured by placing a Doppler\textsuperscript{6} transducer on the shaved palmar surface of a forelimb over the common digital branch of the radial artery to detect blood flow and then placing a blood pressure cuff (width, 40% to 50% of limb circumference) halfway between the elbow and the carpus, with the limb positioned with the elbow and carpal joints in extension. Heart rate and ECG\textsuperscript{7} readings were monitored continuously. Arterial hemoglobin saturation was also monitored continuously with a pulse oximeter.\textsuperscript{8}

**MAC determination**—Approximately 45 minutes after induction of anesthesia, with the ETISO held constant at 2.0% for at least 20 minutes, the baseline ISOMAC was determined by use of a bracketing technique for rabbits.\textsuperscript{20} The noxious stimulus consisted of clamping a pedal digit with 24-cm sponge forceps,\textsuperscript{1} with protective plastic tubing on each forceps jaw. The forceps was closed to the first notch until gross purposeful movement (defined as gross movement of the head or extremities) was detected or a period of 60 seconds elapsed, whichever happened first. Coughing, straining, stiffening, and chewing were not considered purposeful movement. When purposeful movement was detected, the ETISO was increased by 0.1%; otherwise, it was decreased by 0.1%, and the stimulus was reapplied after a 20-minute equilibration period. The order in which the limbs and digits were clamped was randomized.

The ISOMAC was defined as the mean of the 2 ETISO values at which movement was and was not detected. The ISOMAC determination was performed in duplicate, and the mean value was taken as the ISOMAC. However, when the difference between the 2 ISOMAC values was >10%, a third ISOMAC was determined and averaged with the first 2 to attain the ISOMAC.

A 5% tramadol hydrochloride solution was prepared from tramadol hydrochloride powder according to a reported method.\textsuperscript{21} Potency was confirmed with reversed-phase HPLC, as described elsewhere.\textsuperscript{22} After the ISOMAC was determined, tramadol (4.4 mg/kg) made up to a total volume of 1 mL with saline (0.9% NaCl) solution was administered IV over 60 seconds. Determination of ISOMAC\textsubscript{2} began 20 minutes after administration of tramadol, with the ETISO held constant at ISOMAC for at least 20 minutes. The ISOMAC\textsubscript{2} was determined as for the ISOMAC. After the ISOMAC\textsubscript{2} was determined, rabbits were allowed to recover from anesthesia. Interval from termination of isoflurane anesthesia to extubation (minutes) was recorded for each rabbit.

**Drug analysis**—For determination of plasma tramadol and M1 concentrations, a blood sample (approx 4 mL) was collected from a jugular vein immediately after the ISOMAC\textsubscript{2} was determined. Blood samples were placed in lithium heparin tubes, and plasma was harvested and stored at –80°C before analysis.

Measurement of plasma tramadol and M1 concentrations was performed by means of reversed-phase HPLC with fluorescence detection as described elsewhere.\textsuperscript{23} Intra-assay variability ranged from 0.3% to 11.2% for M1 and 0.2% to 3.9% for tramadol. Inter-assay variability ranged from 2.8% to 8.4% for M1 and 1.9% to 9.5% for tramadol.

**Statistical analysis**—All analyses were performed with commercial software.\textsuperscript{8} Percentage change in MAC was calculated by use of the following equation: \((\text{ISOMAC}_{\text{2}} – \text{ISOMAC})/\text{ISOMAC} \times 100\). Values for interval to ISOMAC determination, ISOMAC, interval to ISOMAC, determination, ISOMAC, percentage change in ISOMAC, tramadol concentration, and M1 concentration are reported as mean ± SD. A paired t test was used to evaluate the percentage difference between the ISOMAC and ISOMAC\textsubscript{2}. A mixed-model repeated-measures ANOVA was used to test for significant changes in...
heart rate, blood pressure, and esophageal temperature over time. The variable rabbit was included in the model as a random effect. Results of the ANOVA are reported as the least-squares mean ± SEM. Interval to extubation is reported as mean ± SD. Correlations for interval to ISOMAC, determination, tramadol concentration, and M1 concentration with percentage change in the ISOMAC were analyzed by calculation of the Pearson product-moment correlation. Values of \( P < 0.05 \) were considered significant for all analyses.

**Results**

All 6 rabbits completed the experiment to evaluate the effect of tramadol administration on the ISOMAC. No adverse effects of anesthesia or tramadol administration were detected in rabbits at any time.

Mean ± SD values for the ISOMAC and ISOMAC\(_r\) were 2.33 ± 0.13% and 2.12 ± 0.17%, respectively. The ISOMAC decreased significantly by 9 ± 4% after administration of tramadol (\( P = 0.004 \)). The interval from induction of anesthesia to determination of the ISOMAC was 99 ± 15 minutes, and the interval from determination of the ISOMAC to determination of the ISOMAC\(_r\) was 65 ± 6 minutes. The overall interval to ISOMAC\(_r\) determination ranged from 208 to 250 minutes.

The mean plasma tramadol concentration at the time of ISOMAC\(_r\) determination was 346 ± 152 ng/mL (range, 181 to 636 ng/mL), and that for plasma M1 concentration was 41 ± 12 ng/mL (range, 32 to 61 ng/mL). Intervals to determination of ISOMAC\(_r\), plasma tramadol concentration, and plasma M1 concentration were not correlated with the percentage change in the ISOMAC (\( r = 0.16 \) [\( P = 0.76 \)], \( r = 0.19 \) [\( P = 0.72 \)], and \( r = 0.56 \) [\( P = 0.23 \)], respectively).

Statistical analyses revealed that heart rate decreased significantly (\( P = 0.002 \)) immediately after tramadol administration but was not significantly different from the pretreatment value by 10 minutes after administration (Table 1). The SAP decreased to approximately 60 mm Hg for approximately 5 minutes in 3 rabbits after tramadol administration, but the overall change in value was not significant. Esophageal temperature did not change after tramadol administration. The \( \text{PETCO}_2 \) was 30 to 32 mm Hg and hemoglobin saturation was > 96% at all times. Mean ± SD interval to extubation was 6.7 ± 3.5 minutes (range, 3 to 13 minutes) after discontinuation of isoflurane.

The HPLC analysis revealed that the potency of the tramadol solution was 98%. Mean percentage recoveries from plasma samples were 93% and 84% for M1 and tramadol, respectively.

**Discussion**

The baseline ISOMAC (2.33 ± 0.13%) in the study reported here was slightly higher than the value (2.05 ± 0.18%) reported for New Zealand White rabbits in a study\(^5\) in which a tail-clamping technique was used as the noxious stimulus for MAC determination. Other studies involving New Zealand White rabbits in which the digit-clamping technique was used as the noxious stimulus revealed ISOMAC values of 2.08 ± 0.02%\(^6\) and 2.49 ± 0.07%\(^7\). Interindividual and intraindividual variations in MAC values are typically < 20% and 10%, respectively\(^8\). However, the MAC of an inhalation anesthetic can differ substantially among animals of the same species and even among strains of the same species.\(^9\) Variation within our study was minimized by having 1 observer of purposeful movement (CME) and maintaining esophageal temperature, hemoglobin saturation, \( \text{PETCO}_2 \), and arterial blood pressure within ranges that do not affect the MAC.\(^10\) The rabbits were slightly hypocarbic during MAC determination, but this degree of hypocarbia does not affect the MAC. The transient hypotension (SAP, approx 60 mm Hg) that was evident in 3 rabbits immediately after tramadol administration was unlikely to have affected the MAC. Within a species, the variability of the MAC is not influenced by duration of anesthesia, an SAP > 50 mm Hg, or \( \text{PaCO}_2 \) values between 10 and 90 mm Hg.\(^10\)

In the rabbits of the study reported here, IV administration of tramadol at a dose of 4.4 mg/kg resulted in mean plasma tramadol and M1 concentrations of 346 ng/mL and 41 ng/mL, respectively, and a reduction in ISOMAC of 9%, compared with the value before tramadol administration. This ISOMAC reduction was not correlated with plasma tramadol or M1 concentrations. In rats, IV administration of tramadol (10 mg/kg) significantly reduces the MAC of isoflurane by 16%\(^1\) and in cats, oral administration of tramadol (8.6 to 11.6 mg/kg) reduces the MAC of sevoflurane by 40%.\(^1\) The studies\(^1\) involving dogs, when tramadol was administered via a constant rate IV infusion of 1.3 mg/kg/h or 2.6 mg/kg/h, the resulting plasma concentrations of tramadol (2.201 ± 1.532 ng/mL and 4.446 ± 3.873 ng/mL, respectively) and M1 (57 ± 18 ng/mL and 86 ± 20 ng/mL, respectively) caused a reduction in ISOMAC of 26% to 36% when a noxious electrical stimulus was used, although this change was not correlated with plasma tramadol or M1 concentrations.\(^1\)

In the present study, the variability in plasma tramadol and M1 concentrations at the time of ISOMAC\(_r\) determination and lack of correlation with percentage reduction in ISOMAC could have been attributable to the effects of anesthesia on drug distribution, clearance, and elimination; individual and species variability in pharmacokinetic and pharmacodynamic responses to tramadol; and variability in the interval to ISOMAC\(_r\) determination in the rabbits. Administration of a constant rate infusion of tramadol following the bolus injection may have reduced some of this variability.
Few studies have been conducted to investigate the MAC-reducing effects of analgesics in rabbits. In 1 study in which the effects of diclofenac and ketoprofen in rabbits were evaluated, the MAC of halothane increased after drug administration. In another study, administration of butorphanol alone or with meloxicam significantly reduced the ISOMAC in rabbits by 7.6% to 12.4%. The MAC-reducing effects of tramadol could be attributable to activation of opioid, serotoninergic, or α-adrenergic receptors by the parent drug or any of its metabolites. Activation of opioid, serotoninergic, and noradrenergic receptors reportedly mediates analgesia in humans, dogs, and rats. Interestingly, in a model of peripheral neuropathy in rats, the analgesic effects of tramadol appeared to change from opioid receptor mediated to α-adrenergic receptor mediated over time. Opioid receptor activation is an important mechanism for the reduction in MAC achieved with tramadol in rats and cats because naloxone blocks its MAC-reducing effect in those species. The M1 metabolite reportedly has a considerable analgesic effect in humans attributable to its action at opioid receptors, but there are several tramadol metabolites that could be responsible for its ISOMAC-lowering effects. Measurement of the reduction in ISOMAC achieved with tramadol after treatment with opioid, α-adrenergic, or serotonin receptor antagonists would help to determine the mechanism of the ISOMAC reduction in rabbits.

Although pharmacokinetic characteristics and antinociceptive properties of tramadol have now been reported for cats and dogs, few studies have evaluated minimum effective doses for analgesia have not been determined. The pharmacokinetic and pharmacodynamic characteristics of tramadol when administered IV have not been evaluated in rabbits, and the dose used in the present study was chosen on the basis of a published dose of tramadol that appears safe in awake dogs. Although there were clinically unimportant decreases in the ISOMAC in our study, there were significant decreases in heart rate and, although transient, a significant decrease in SAP in 3 of 6 rabbits after tramadol was administered over 1 minute, indicating that higher doses of tramadol may cause more significant cardiovascular depression. Slower IV administration of tramadol, over 5 minutes rather than over 1 minute, might have reduced the adverse cardiovascular effects detected in the rabbits. Besides providing preemptive analgesia, preemptive administration of analgesics often has a MAC-sparing effect, allowing lower concentrations of volatile anesthetic to be used to achieve surgical anesthesia, resulting in an improvement in cardiopulmonary variables. Even a modest decrease in the ISOMAC can result in improved cardiac output and tissue perfusion.

References

23. Drummond J. MAC for halothane, enflurane, and isoflurane in...