FULL PAPER *Surgery*

Effects of a Single Bolus Intravenous Dose of Tramadol on Minimum Alveolar Concentration (MAC) of Sevoflurane in Dogs

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ABSTRACT. Tramadol is an atypical opioid analgesic widely used in small animal practice. This study was designed to determine the effect of a single intravenous (IV) dose of tramadol on the minimum alveolar concentration (MAC) of sevoflurane in dogs. Six beagle dogs were anesthetized twice to determine the sevoflurane MAC with or without an administration of tramadol (4 mg/kg, IV) at 7 days interval. The sevoflurane MAC was determined using a tail clamp method in each dog ventilated with positive pressure ventilation. The tramadol administration produced a significant reduction in the sevoflurane MAC by 22.3 ± 12.2% (1.44 ± 0.28% with tramadol versus 1.86 ± 0.30% without tramadol, P=0.010). This MAC reduction had been determined from 122 ± 19 to 180 ± 41 min following the tramadol administration. During this period, the plasma concentrations of tramadol and its metabolite, O-desmethyltramadol (M1), decreased from 429 ± 64 to 332 ± 55 *n*g/m*l* and from 136 ± 24 to 114 ± 68 *n*g/m*l*, respectively, but these changes were not statistically significant. There was no significant difference in heart rate, mean arterial blood pressure and SpO_2 between the control and tramadol treatment. The dogs that received tramadol treatment sometimes breathed spontaneously. Therefore, their respiratory rates significantly increased, and $PETCO_2$ decreased during the MAC determination. In conclusion, the single IV dose of tramadol produced a significant reduction in the sevoflurane MAC in dogs.

KEY WORDS: canine, minimum alveolar concentration (MAC), O-desmethyltramadol (M1), sevoflurane, tramadol.

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Preemptive analgesia (i.e., treatment using analgesic drugs before pain occurs) reduces the amount of anesthetic and analgesic drugs required for producing surgical anesthesia and controlling postoperative pain, helps to stabilize anesthesia and decreases overall patient morbidity associated with surgery and anesthesia [19]. Opioid produces a strong analgesia by acting on one or more specific opioid receptors in the brain and spinal cord [20]. Preemptive opioid administration decreases the amount of volatile anesthetic agent required to produce general anesthesia [21].

Tramadol is a centrally acting 'atypical' opioid analgesic and produces a synergistic analgesic effect provided by a μ-opioid receptor affinity coupled with inhibitions of synaptic reuptake of monoamine neurotransmitters, such as 5-hydroxytryptamine (5-HT) and norepinephrine [8, 24]. Tramadol has gained popularity in small animal practice, because it is perceived to be an effective analgesic without tough control by law. Preemptive administration of tramadol control postoperative pain after ovariohysterectomy without significant adverse effects in dogs [14, 17]. One of the metabolites from tramadol, O-desmethyltramadol (M1), also has a weak μ agonist effect and has up to 200 times more μ-opioid receptor binding activity than tramadol [8, 9, 24]. M1 may be playing a part of the anti-nociceptive effects of tramadol, because its production from the parent compound has been demonstrated in dogs [15, 29].

Sevoflurane is a volatile anesthetic drug with a relatively low blood/gas solubility coefficient resulting in rapid induction and recovery from anesthesia [27]. Because of these strong points, sevoflurane has become a popular inhalation anesthetic in veterinary practice. However, it should be remembered that sevoflurane causes dose-dependent hypotension, hypoventilation, impaired cardiac contractility and hypothermia in dogs [27]. On the other hand, it has been reported that tramadol is a mild myocardial depressant in dogs anesthetized with sevoflurane [10]. A sparing effect on anesthetic requirement provided by the preemptive administration of tramadol is expected to convey the advantage of preserving cardiovascular function in patients anesthetized with sevoflurane. Therefore, it is important for veterinary practitioners to confirm the effect of tramadol on the sevoflurane requirement in dogs.

Seddighi *et al*. [26] reported that constant rate infusion (CRI) of tramadol reduced the minimum alveolar concentration (MAC) of sevoflurane, but this was not dose dependent in dogs. On the other hand, it was reported that the minimum effective plasma concentration of tramadol as calculated for humans had been maintained for about 6–7 hr following an intravenous administration (IV) of tramadol in dogs [6].

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We hypothesized that a single IV dose of tramadol might produce a useful sparing effect on the MAC of inhalation anesthetics. However, there is no published report evaluating effects of a single IV dose of tramadol on the MAC in dogs. The purpose of the study reported here was to evaluate the sparing effect of a single IV dose of tramadol on the sevoflurane MAC in dogs.

MATERIALS AND METHODS

Experimental animals: Six beagle dogs aged from 8 to 10 years (9.7 ± 0.8 years mean ± SD, comprising 3 males and 3 females) and weighing from 8.8 to 15.5 kg (11.6 ± 2.4 kg) were used for this study. The dogs were judged to be in good to excellent health based upon the results of a physical examination, complete blood cell count and serum biochemical analysis. Food and water were withheld from dogs for 12 hr before the commencement of each experiment. The dogs were anesthetized for the determination of sevoflurane MAC on two occasions using a randomized crossover study design, with a 7 days interval between treatments. For each anesthetic occasion, the dogs received a single bolus IV of 4 mg/kg tramadol (Tramal, Nippon Shinyaku Co., Kyoto, Japan) (TRM treatment; n=6) or not (control; n=6). The dogs were cared for according to the principles of the "Guide for the Care and Use of Laboratory Animals" prepared by Rakuno Gakuen University. The Animal Care and Use Committee of Rakuno Gakuen University approved the study.

Anesthesia and instrumentation: Anesthesia was induced by mask induction using sevoflurane (Sevoflo, Dainippon-Sumitomo Pharma, Osaka, Japan) in oxygen, a 33-Fr cuffed endotracheal tube (Phycon, Fuji Systems Co., Tokyo, Japan) was positioned into the trachea and the dogs were placed in right lateral recumbency. Anesthesia was maintained with sevoflurane in oxygen (2 *l*/min) delivered via a circle anesthetic rebreathing system (Beaver 20, Kimura Medical Instrument Co., Tokyo, Japan) with an out-of-circuit vaporizer (Sevotech III, Ohmeda, Datex-Ohmeda, Tokyo, Japan). The end-tidal partial pressure of CO_2 ($PETCO_2$) was maintained between 35 and 40 mmHg by positive pressure ventilation using a time-cycled ventilator (Nuffield Anesthesia Ventilator Series 200, Penlon, Abingdon Oxon, U.K.). A 22-gauge catheter (Happycath Z, Medikit Co., Tokyo, Japan) was placed in the left cephalic vein and an 18-gauge catheter (Happycath Z, Medikit Co.) was placed into the left jugular vein. The dogs were administered lactated Ringer's solution (Solulact, Terumo, Tokyo, Japan) at an IV infusion rate of 10 m*l*/kg/hr through the 22-gauge catheter. Esophageal temperature was maintained between 37.5 and 38.5°C, using a heating pad (Micro-Temp II, Cincinnati Sub-Zero Products, Cincinnati, OH, U.S.A.) and a warm air blanket (FK-CL3, Sanyo Electric, Moriguchi, Japan).

Anesthesia monitoring: During anesthesia, esophageal temperature (°C), heart rate (beats/min), cardiac rhythm, respiratory rate (breaths/min), $PETCO_2$ (mmHg), end-tidal concentration of sevoflurane (ETSEV;%), indirect blood pressure (mmHg) and saturation of hemoglobin with oxygen (SpO_2;%) were monitored using a veterinary patient monitoring system (BP-508V, Omron Colin Co., Tokyo, Japan). Esophageal temperature was measured using an electric thermometer probe placed orally into the thoracic esophagus. Heart rate and cardiac rhythm were monitored by visual inspection of Lead II of the electrocardiogram. Blood pressure was determined by the oscillometric method. SpO_2 was measured by pulse oximetry. A commercially available adaptor (Airway adaptor L-shape, Omron Colin Co.) modified with an 8-Fr feeding tube (Safeed feeding tube, Terumo) was placed at the Y-piece of the breathing circuit. The tube passed through the endotracheal tube so that its tip rested in the thoracic portion of the trachea. A side-stream capnograph and anesthetic agent monitor was used to determine respiratory rate, $PETCO_2$ and ETSEV. The anesthetic agent monitor was calibrated immediately prior to each experiment, using a calibration kit (AG calibration gas and adaptor set, Omron Colin Co.).

Determination of sevoflurane MAC: The sevoflurane MAC was determined by use of the tail clamp method [13, 30]. Following the instrumentation, the dogs were treated with or without a single bolus IV of 4 mg/kg tramadol through the 22-gauge catheter. Then, each dog was allowed to equilibrate for 30 min at 2.4% of ETSEV. The hair was clipped from a section of the dog's tail with a diameter approximately equivalent to the diameter of a standard Backhaus towel clamp (Backhaus Towel Clamp, Mizuho, Tokyo, Japan). After the equilibration, the towel clamp was then placed around the dog's tail and closed to the third ratchet. The clamp had been left in place for 60 sec or until the dog showed any gross purposeful movement. The purposeful movement was defined as substantial movement of the head or extremities and did not include coughing, chewing, swallowing, or an increasing respiratory effort. The clamp circumscribed the tail and did not puncture the skin of the dog thereby producing a blunt force on the tail [13, 30].

If the dog exhibited any purposeful movement in response to the tail clamping, the ETSEV was increased by 10–20%. If the dog did not exhibit any purposeful movement in response to the tail clamping, the ETSEV was reduced by 10–20%. The dog was retested after 20 min of re-equilibration at the resetting ETSEV. The testing continued until the lowest ETSEV at which the dog did not demonstrate purposeful movement in response to the tail clamping was determined. The MAC was calculated as the mean of the ETSEV at which the dog did not demonstrate any purposeful movement and next lower concentration tested (i.e., the highest concentration at which the dog still demonstrated purposeful movement in response to the tail clamping). The MAC for each dog was determined in triplicate by the same person (K.Y.).

Collection of cardio-respiratory data and plasma samples: Cardio-respiratory data were collected immediately before the MAC determinations. Data from 3 observations recorded immediately prior to tail clamping that produced changes in response to stimulation were obtained from each dog. At the same time, blood samples (2 m*l*) to analyze plasma concentrations of tramadol and M1 were also collected through the 18-gauge catheter placed into the left jugular vein and mixed with heparin sodium (30 units per 1 m*l* of blood) from the

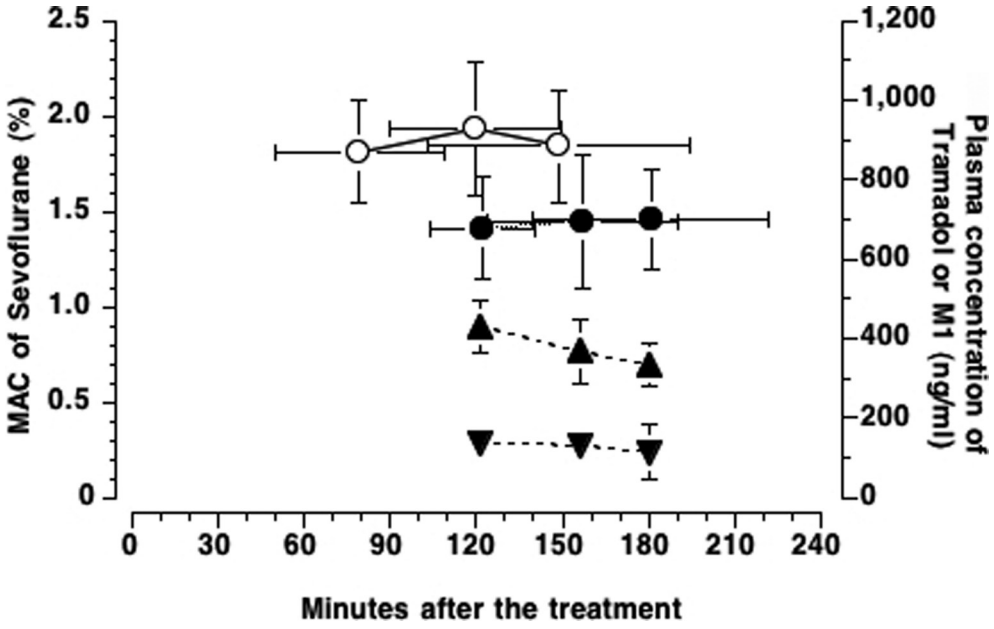

Fig. 1. Minimum alveolar concentration (MAC) of sevoflurane and its corresponding plasma concentrations of tramadol and O-desmethyltramadol (M1) following a single bolus intravenous injection (IV) of tramadol 4 mg/kg in dogs. The MAC was determined in triplicate following an equilibration for 30 min at 2.4% of end-tidal sevoflurane concentration. Plots and error bars represent mean values and standard deviations for 6 dogs, respectively. The administration of tramadol significantly decreased the MAC, compared to control (1.86 ± 0.30% versus 1.44 ± 0.28%, P=0.010). It took 148 ± 45 and 180 ± 41 min to obtain the triplicate data for determination of sevoflurane MAC in dogs with (●) and without (○) a treatment of single bolus IV of tramadol, respectively. During the period for the determination of sevoflurane MAC in triplicate, the mean plasma concentrations of tramadol (▲) and M1 (▼) decreased from 429 ± 64 to 332 ± 55 *n*g/m*l* and 136 ± 24 to 114 ± 68 *n*g/m*l*, but without statistical significance.

dogs that received TRM treatment. The blood samples were immediately centrifuged (1,000 × *g* for 10 min) to separate plasma. The plasma samples were stored at −80°C until analysis.

Measurement of plasma concentrations of tramadol and M1: Each plasma sample (200 *μl*) was mixed with 100% methanol (400 *μl*), and the top clear layer (300 *μl*) was obtained by centrifugation (1,400 × *g* for 5 min). Another 100% methanol (400 *μl*) was mixed with the precipitate, and the top clear layer (300 *μl*) was also obtained by centrifugation. These 2 layers were combined in a tube as an extract. The extract (200 *μl*) was mixed with purified water (600 *μl*), and filtrated with protein precipitation filter (HLK-DISC for ion-chromato, Kanto Kagaku, Tokyo, Japan) and stored at −80°C until high performance liquid chromatography (HPLC) analysis.

The plasma concentration of tramadol and M1 were determined by HPLC consisting of dual pump (DP-8020, Toso, Tokyo, Japan), auto-sampler (AS-8020, Toso), reversed-phase column (Unison UK-C18, Intakt, Kyoto, Japan), integration software (LC-8020, Toso), degasser (AG-12, M&S Instruments, Osaka, Japan) and intelligent fluorescence detector (FS-8020, Toso). Tramadol within each extract sample was separated with the reversed-phase column using a linear gradient mobile phase from methanol-water-ammonium acetate (24:75.94:0.06) to 100% methanol delivered at 0.3 m*l*/min and detected by the fluorescence detector set at 270 nm (excitation) and 304 nm (emission). M1 within each extract sample was also separated with the same column using a linear gradient mobile phase from methanol-water-ammonium acetate (5:94.94:0.06) to 100% methanol delivered at 0.3 m*l*/min and detected by the same sets. Standard curves for the plasma analysis were prepared by diluting tramadol (Tramal, Nippon Shinyaku Co., Kyoto, Japan) and M1 (Sigma-Aldrich, Tokyo, Japan) with untreated canine plasma. The diluted standards were treated exactly as plasma sample. The limits of detection were 5.0 *n*g/m*l* for tramadol and 5.0 *n*g/m*l* for M1.

Statistical analysis: Data are reported as mean ± standard deviation. The cardio-respiratory data and sevoflurane MAC were compared between control and TRM treatment. The percentage MAC reduction was calculated as: percentage MAC reduction=(MAC of control − MAC of TRM treatment)/ MAC of control × 100. These data were analyzed by use of paired *t*-test. Changes in the plasma concentrations of tramadol and M1 were analyzed by use of one-way factorial ANOVA. The level of significance was set at P<0.05.

RESULTS

Sevoflurane MAC and plasma concentrations of tramadol and M1: Figure 1 showed the sevoflurane MAC and its corresponding plasma concentrations of tramadol and M1. The sevoflurane MAC was 1.86 ± 0.30% for the control dogs and 1.44 ± 0.28% for the dogs that received TRM treatment. The triplicate data for the MAC determination were collected at 122 ± 19, 156 ± 33 and 180 ± 41 min after the tramadol administration for the dogs that received TRM treatment. The TRM treatment produced a significant reduction in the MAC (P=0.010), and the percentage MAC reduction was 22.3 ± 12.2%. During the sevoflurane MAC determination, the mean plasma concentrations of tramadol, and M1 decreased gradually from 429 ± 64 to 332 ± 55 *n*g/m*l* and from 136 ± 24 to 114 ± 68 *n*g/m*l*, respectively. However, these changes in plasma concentrations were not statistically significant.

Cardio-respiratory parameters: Cardio-respiratory parameters recorded at the MAC determination are summarized in Table 1. Normothermia was achieved in all dogs throughout the MAC determinations. There was no significant difference in heart rate, mean arterial blood pressure and SpO_2 between the control and TRM treatment. The dogs that received TRM treatment sometimes breathed spontaneously during the MAC determination. Therefore, their respiratory rates significantly increased (P=0.005). In these dogs, $PETCO_2$ decreased significantly (P=0.001) consistent with the increase in respiratory rate.

Table 1. Cardio-respiratory parameters recorded at the determination of sevoflurane MAC

	Control dogs	Dogs received tramadol
Esophagus Temperature (°C)	38.0 ± 0.2	38.0 ± 0.2
Heart Rate (beats/min)	102.9 ± 15.6	101.5 ± 31.9
IMABP (mmHg)	86.3 ± 9.2	87.0 ± 16.6
SpO_2 (%)	97.5 ± 1.4	97.2 ± 1.0
Respiratory Rate (breaths/min)	12.1 ± 0.4	28.3 ± 21.4**
$PETCO_2$ (mmHg)	36.7 ± 1.1	32.4 ± 5.4**

Data were shown in mean ± standard deviation for 18 observations from 6 dogs. Data from 3 observations recorded immediately prior to tail clamping that produced changes in response to stimulation were obtained from each dog. IMABP: indirect mean arterial blood pressure, SpO_2: saturation of hemoglobin with oxygen, $PETCO_2$: end-tidal partial pressure of CO_2. Significant difference from the control dogs detected by paired *t*-test: **P<0.01.

DISCUSSION

We adopted the IV dose of 4 mg/kg tramadol based on previous reports on pharmacokinetics of an IV injection of tramadol [15, 18], a clinical study [17] and our clinical investigations in dogs. In the present study, a single bolus IV dose of 4 mg/kg tramadol produced a significant decrease in the sevoflurane MAC by 22.3 ± 12.2% following the tramadol administration without clinically significant depression in cardiopulmonary functions. Therefore, a single IV dose of tramadol may have clinical benefits as a part of premedication in dogs. However, there is a limitation on the present study, because our data were collected from comparatively older dogs.

In the present study, the sevoflurane MAC of the control dogs was 1.86 ± 0.30%, which is lower compared with sevoflurane MAC reported by Kazama *et al.* [12] for a larger group of unpremedicated young dogs (2.36 ± 0.46%, n=18, aged 10 to 19 months). The six dogs used in the present study were aged 8 to 10 years. It was reported that the sevoflurane MAC was reduced with aging up to approximately 17% in the dogs aged 9 years compared to those in the dogs aged 2 years [30]. Therefore, the age of dogs is considered a primary factor in the lowering of sevoflurane MAC of the control dogs.

The potency of inhalation anesthetics is evaluated by use of the concept of MAC, which is the alveolar concentration of an anesthetic at which 50% of the population dose not respond with purposeful movement to a noxious stimulus [27]. The sparing effect of analgesics on the MAC of inhalation anesthetics also provides several reliable and quantifiable variables that allow comparison between different analgesic drugs [2]. In this present study, a single bolus IV dose of 4 mg/kg tramadol produced a significant decrease in the sevoflurane MAC by 22.3% for dogs aged from 8 to 10 years old. A similar reduction in MAC with the use of tramadol might be achieved by administration of commonly used injectable drugs. For example, morphine, a potent opioid receptor agonist, produced a dose-dependent reduction of enflurane MAC (17% for 0.5 mg/kg, 32% for 2 mg/kg, 63% for 7 mg/kg and 67% for 27 mg/kg IV) in dogs [21]. Butorphanol, an agonist- antagonist opioid, also produced a significant reduction of enflurane MAC, but no further reduction in enflurane MAC occurred at higher dosage (11% for 0.1 mg/kg, 15% for 0.4 mg/kg, 15% for 1.4 mg/kg and 16% for 5.4 mg/kg IV) in dogs [21]. Therefore, a single IV dose of 4 mg/kg of tramadol may be expected to produce a sparing effect on MAC of sevoflurane in older dogs.

Seddighi *et al*. [26] reported that tramadol infusion reduced the sevoflurane MAC, but this was not dose dependent in dogs. In the report, tramadol infusion at a rate of 1.3 mg/kg/hr CRI following 1.5 mg/kg IV decreased the sevoflurane MAC by 26%, and its plasma concentration at the MAC determination was 2,873 ± 2,474 *n*g/m*l* [26]. Interestingly, the single IV dose of 4 mg/kg tramadol produced an equivalent reduction of sevoflurane MAC with a quite lower plasma concentration of tramadol (332 ± 55 *n*g/m*l*) in the present study. Although the minimum effective plasma concentration of tramadol for dogs has not been reported, it should be lower than 332 *n*g/m*l* for tramadol judging from the result of the present study. In humans, it was reported that the minimum effective plasma concentrations of tramadol was 287.7 *n*g/m*l* [16]. Giorgi *et al*. [6] reported that the minimum effective plasma concentration of tramadol as calculated for humans had been maintained for about 6–7 hr following a single IV administration of 4 mg/kg tramadol in dogs. We previously reported [10] that the plasma concentration of tramadol gradually decreased over time from approximately

2,000 *n*g/m*l* at 5 min to 500 *n*g/m*l* at 120 min after a single IV dose of 4 mg/kg tramadol in older dogs (8–10 years old) anesthetized with sevoflurane 1.3 MAC. In these dogs, the plasma concentration of M1 showed a peak (332 *n*g/m*l*) at 15 min and then gradually decreased to approximately 200 *n*g/m*l* at 120 min after the tramadol administration. The concentrations of tramadol and M1 at 120 min after the single IV dose of 4 mg/kg tramadol were almost equivalent between our previous study [10] and the present study. The sparing effect of tramadol on the sevoflurane MAC had been maintained during the period from 122 ± 19 to 180 ± 41 min following the single IV dose of 4 mg/kg tramadol. Therefore, a single IV dose of 4 mg/kg tramadol is expected to provide a significant sparing effect on the sevoflurane MAC lasting for at least 3 hr following the administration in older dogs.

Some authors, especially in human studies, use the plasma concentration of M1 to determine the analgesic effect of tramadol, because the evidence indicates that M1 is responsible for most of the therapeutic effects rather than the parent drug [4]. Giorgi *et al*. [6] stated that M1 might not be responsible for the major clinical effectiveness in dogs, because M1 was detected at a lower concentration than the minimum effective plasma concentration (40 *n*g/m*l*) reported in humans [7]. However, a higher M1 concentration (65 ± 16 *n*g/m*l*) was detected in dogs infused tramadol at a rate of 1.3 mg/kg/hr CRI following 1.5 mg/kg IV [26]. We also detected M1 at a higher plasma concentration (114–136 *n*g/m*l*). Further investigation will be necessary to confirm the sparing effect of M1 on the sevoflurane MAC in dogs administered tramadol.

Tramadol elimination rate is rapid in awaking dogs [5, 15, 18]. The plasma concentrations of tramadol and M1 in our dogs were a little higher than those in awaking dogs [15, 18]. Tramadol is metabolized in the liver, and its metabolites primarily were excreted via the kidneys (90%) with the remaining (10%) being excreted via feces [25, 29]. In addition, volatile inhalation anesthetics including sevoflurane cause a dose-dependent cardio-pulmonary depression in dogs [27]. It was also reported that portal venous blood flow was reduced with sevoflurane anesthesia in dogs [3]. Therefore, it was suggested that the higher plasma concentrations of tramadol and M1 in our dogs might result from decreases in the metabolism of tramadol and elimination of its metabolites caused by sevoflurane anesthesia. However, there is a limitation on the present study, because our data were collected from comparatively older dogs. Generally, geriatric animals decrease renal and hepatic tissue mass, drug metabolism and drug clearance [23]. Further investigation will be necessary to confirm the effect of sevoflurane anesthesia on the metabolism of tramadol in dogs.

In the present study, there was no significant difference in heart rate, mean arterial blood pressure and SpO_2 between the control and TRM treatment. On the other hand, the dogs treated with tramadol sometimes breathed spontaneously and showed significant increase in respiratory rate and decrease in $PETCO_2$, compared with the control dogs. Tramadol (4 mg/kg IV) with sevoflurane 1.3 MAC produced a mild increase in arterial blood pressure for only 15 min after administration [10]. In addition, tramadol does not cause respiratory depression in humans [1, 28]. Tramadol (5 to 20 mg/kg IV) did not cause respiratory depression and clearly increased respiratory rate and volume in anesthetized dogs [22]. Sevoflurane produces a dose-dependent respiratory depression in dogs [27]. The dogs treated with tramadol were anesthetized with somewhat lower sevoflurane concentrations during the MAC determination, compared to the control dogs. The MAC is determined based on the immobility in response to painful stimulation and may reflect the suppression of motor neurons at the ventral horn in the spinal cord [11]. Increasing nociceptive stimulation and lower sevoflurane concentration during the tail clamping might cause the increase in respiratory rate in the dogs treated with tramadol. However, all cardio-respiratory data were recorded with no stimulation before each tail clamp following the 20-min equilibration. In addition, there was no significant difference in heart rate and arterial blood pressure between groups. Therefore, the increase in respiratory rate seems not to be responsible for the increasing nociceptive stimulation. The increase in respiratory function observed in the dogs treated with tramadol might be caused by the lower sevoflurane concentration during the MAC determination and/or respiratory stimulation of tramadol. It is expected that the administration of tramadol may overcome the respiratory depression developed in dogs anesthetized with sevoflurane.

In conclusion, a single IV dose of 4 mg/kg of tramadol produced a significant reduction in the sevoflurane MAC lasting for at least 3 hr following the administration in dog. In addition, cardiovascular and respiratory depressions induced by volatile inhalation anesthetics during anesthesia is expected to be relieved by tramadol for its sparing effect on the sevoflurane MAC and less cardio-respiratory effect. A single IV dose of tramadol may have clinical benefits as a part of premedication in dogs.

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