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Influence of sevoflurane anesthesia with mechanical ventilation and fluid-therapy on distribution of subcutaneously administered robenacoxib in dogs

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ABSTRACT. Robenacoxib is a novel nonsteroidal anti-inflammatory drug approved for dogs. The present study aimed to evaluate influences of sevoflurane anesthesia on the distribution of robenacoxib in dogs. Ten healthy beagle dogs (1 to 11 years old, 9.3 to 14.3 kg body weight, 6 males and 4 females) were subcutaneously administered robenacoxib (2 mg/kg) under conscious condition or sevoflurane anesthesia inhaled a 1.3-fold predetermined individual minimum alveolar concentration of sevoflurane at a 28-day interval. The dogs under sevoflurane anesthesia were also mechanically ventilated and received fluid-therapy. On each occasion, serum samples were collected from the dogs before and at 5, 15, 30, 60, 120, 180, and 240 min after the robenacoxib administration. Serum robenacoxib concentration was measured by a liquid chromatography-tandem mass spectrometry. Maximum serum concentration of robenacoxib (C_{max}) was 2.2 μg/ml [range: 1.2–4.6] (median [range: minimum-maximum]) and time of C_{max} (T_{max}) was 90 min [range: 60–120] in the conscious dogs. In the sevoflurane-anesthetized dogs, the C_{max} significantly declined (1.3 μg/ml [range: 0.8–1.4], P=0.008) and T_{max} was delayed (120 min [range: 120–240], P=0.018) compared with those in the conscious dogs. The serum robenacoxib concentration at 240 min (C_{240}) decreased to 0.5 μg/ml [range: 0.2–0.9] in the conscious dogs, while it remained higher in the sevoflurane-anesthetized dogs (1.0 μg/ml [range: 0.3–1.4], P=0.011). In conclusion, the anesthetic procedure with sevoflurane, mechanically ventilated, and received fluid-therapy might affect the pharmacokinetics of subcutaneously administered robenacoxib in dogs.

KEY WORDS: dog, pharmacokinetics, robenacoxib, sevoflurane

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Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used in veterinary practice for control of pain and inflammation associated with acute and chronic disease, especially orthopedic disorders and surgery. These drugs affect the arachidonate cascade associated with inflammatory responses and, consequently, inhibit cyclooxygenase (COX) activity, resulting in anti-inflammatory and analgesic effects [15]. Two primary forms of COX have been identified such as COX-1 and COX-2 [15, 24]. Initially, COX-1 was identified as a constitutive isoform mainly responsible for synthesis of physiological prostanoids, which serve to protect various organs, whereas COX-2 was identified as an inducible isoform responsible for inflammatory responses [15], but further studies have shown that both isoforms are constitutive and inducible [16, 27, 28].

Robenacoxib is a novel member of the coxib group of NSAIDs that strongly inhibits COX-2 [4, 13, 14] and it has been demonstrated to produce analgesic effects with minimal side effects in dogs [6, 7, 12, 21, 22]. Robenacoxib has several favorable pharmacokinetic properties including a short time to achieve a maximum blood concentration and a high bioavailability after a subcutaneous administration in dogs [11]. Owing to its acidic nature, robenacoxib is highly bound to plasma proteins with the result that it concentrates in inflamed tissues such as an arthritic joint [23], thereby displaying the property of tissue selectivity [5]. Preoperative administration of robenacoxib has been demonstrated to be effective for control of pain and inflammation associated

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with soft-tissue and orthopedic surgeries in dogs [8–10]. Furthermore, it has been reported that a subcutaneous administration of robenacoxib (2 mg/kg) decreased the minimum alveolar concentration (MAC) for blunting adrenergic response (MAC-BAR) of sevoflurane to approximately 17% in dogs [26]. Therefore, the pre-anesthetic medication with robenacoxib is considered feasible in terms of safety and efficacy for anesthesia and analgesia in dogs. In fact, the preoperative subcutaneous administration of robenacoxib to dogs has been approved in some countries including Japan.

In current veterinary practice, volatile anesthetics are widely used for maintenance of anesthesia in many species. Sevoflurane is a volatile anesthetic that has a property of low blood/gas solubility resulting in rapid anesthetic induction and recovery, and faster control of depth of anesthesia than isoflurane [19]. On the other hand, it is reported that the pharmacokinetics of drugs are affected due to the altered cardiac output and regional blood flow distribution caused by volatile anesthetics in dogs [1, 2]. Thus, the pharmacokinetics of drugs administered as pre-anesthetic medication may be affected during the maintenance of anesthesia using volatile anesthetics. In particular, the distribution of subcutaneously administered drugs seems to be strongly affected by volatile anesthetics because the drug-absorption from the site of administration may also be affected due to altered cardiac output and regional blood flow distribution as well as the distribution of drugs [1, 2, 19].

Although the pharmacokinetic properties of robenacoxib in the conscious dogs have been reported in detail [11], as far as we know, there is no study regarding pharmacokinetic properties of robenacoxib in dogs under inhalation anesthesia. Therefore, the purpose of present study was to evaluate the influence of sevoflurane anesthesia on the distribution of subcutaneously administered robenacoxib in dogs. We hypothesized that sevoflurane anesthesia would affect the changes in the distribution of robenacoxib in dogs.

MATERIALS AND METHODS

Experimental animals

Ten intact healthy beagle dogs (1 to 11 years old, 9.3 to 14.3 kg body weight, 6 males and 4 females) were used for 3 experiments with 28-day intervals. On the first occasion, a minimum alveolar concentration (MAC) of sevoflurane was determined by the tail clamp method [29] in each dog. On the second occasion, robenacoxib was administered subcutaneously and animals were assigned in random order using a table of binary random numbers to two different groups: under conscious condition or sevoflurane anesthesia inhaled a 1.3-fold predetermined individual sevoflurane MAC. On the third occasion, robenacoxib was administered subcutaneously and animals were allocated to the other group to which they were included in the second anesthesia. The dogs were judged to be in good to excellent health based upon a physical examination, blood cells count, serum biochemical determination, and chest radiography. Food was withheld from the dogs for 12 hr before each experiment, but allowed free access to water. 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 present study (Approval No. VH14B6).

Robenacoxib administration under conscious condition

In each dog, the right cervical area for placement of central venous catheter was locally desensitized with 1 m*l* of 2% lidocaine (2% Xylocaine: AstraZeneca, Osaka, Japan) and aseptically prepared. Then, an 18-guage central venous catheter (Vascular Indwelling Catheter Kit, Medikit Co., Ltd., Tokyo, Japan) was placed into the right jugular vein. The dogs rested for one hr after the placement of central venous catheter and then received a subcutaneous injection of robenacoxib (2 mg/kg; Onsior injection, Novartis Animal Health Inc., Tokyo, Japan).

Robenacoxib administration under sevoflurane anesthesia

In all the dogs, anesthesia was induced by mask using sevoflurane (Sevoflo, DS Pharma Animal Health Co., Ltd., Osaka, Japan) vehiculated in 100% oxygen. The dogs were orotracheally intubated after the induction and anesthetized with oxygen and a 1.3-fold of individual sevoflurane MAC in left lateral recumbency. The dogs were catheterized using a 22-guage catheter (Supercath, Medikit Co., Ltd., Tokyo, Japan) into the right cephalic vein and intravenously administered lactated Ringer's solution (Solulact, Terumo Co., Ltd., Tokyo, Japan) at a rate of 5 m*l*/kg/hr. The right cervical area for placement of central venous catheter was locally desensitized with 1 m*l* of 2% lidocaine (2% Xylocaine: AstraZeneca) and aseptically prepared. Then, an 18-guage central venous catheter (Vascular Indwelling Catheter Kit, Medikit Co., Ltd.) was placed into the right jugular vein. At 20 min after the completion of instrumentation, the dogs received a subcutaneous administration of robenacoxib (2 mg/kg) and anesthesia was maintained with a 1.3-fold of individual MAC of sevoflurane for 240 min.

During the experiment, the end-tidal partial pressure of CO_2 ($PETCO_2$) was maintained between 35 and 40 mmHg by intermittent positive pressure ventilation (IPPV) using a time-cycled ventilator (Nuffield Anesthesia Ventilator Series 200, Penlon, Abingdon, UK). Esophageal temperature was measured in the dogs using an electric thermometer probe (701J-L5 9Z46, Omron Colin, Ltd., Tokyo, Japan) orally placed into the thoracic esophagus and was maintained between 37.5 and 38.5°C using a heating pad and a warm air blanket. Esophageal temperature (°C), heart rate (beats/min), electrocardiographic readings (monitored through lead II), respiratory rate (breaths/min), mean arterial blood pressure measured by the oscillometric method (MABP; mmHg), hemoglobin oxygen saturation measured by pulse oximetry (SpO_2; %), $PETCO_2$ (mmHg), and end-tidal concentration of sevoflurane (ETsev; %) were recorded every 5 min using a veterinary patient monitoring system (BP-608V, Omron Colin, Ltd.).

Table 1. Changes in cardio-respiratory measurements in 10 sevoflurane-anesthetized dogs

	Minutes after robenacoxib administration					
	0	30	60	120	180	240
Esophageal temperature (°C)	38.0 [37.7–38.4]	38.1 [37.8–38.2]	38.2 [37.7–38.5]	38.1 [37.8–38.5]	38.1 [37.9–38.5]	38.1 [37.8–38.5]
Heart rate (beats/min)	112 [82–124]	112 [80–130]	114 [81–132]	113 [76–140]	114 [84–144]	114 [66–137]
Respiratory rate (breaths/min)	12	12	12	12	12	12
SpO_2 (%)	99 [95–100]	99 [96–100]	99 [97–100]	99 [97–100]	99 [96–100]	99 [98–100]
$PETCO_2$ (mmHg)	37 [36–41]	36 [35–39]	38 [35–40]	37 [35–40]	36 [35–39]	36 [35–38]
MABP (mmHg)	64 [60–77]	69 [62–80]	81 [66–96]	81 [68–95]	78 [64–118]	79 [61–87]

Cardio-respiratory measurements were recorded every 5 min during the sevoflurane anesthesia. Data showed the median [range] at 0, 30, 60, 120, 180, and 240 min after the induction from 10 dogs anesthetized with sevoflurane. SpO_2: hemoglobin oxygen saturation measured by pulse oximetry. $PETCO_2$: the end-tidal partial pressure of CO_2. MABP: mean arterial blood pressure measured by the oscillometric method.

Serum sample collections and measurement of serum robenacoxib concentration

In both groups of dogs, venous blood samples (4 m*l*) were collected from the central venous catheter before and at 5, 15, 30, 60, 120, 180, and 240 min after the administration of robenacoxib. The blood samples were transferred into serum-separation tubes (Neotube, NIPRO, Co., Ltd., Osaka, Japan). Then, serum was separated from the each blood sample by centrifugation (3,000 rpm for 10 min) at room temperature using a centrifuge (KN-70, KUBOTA Co., Ltd., Tokyo, Japan) and stored at −80°C until measuring robenacoxib concentration.

Serum robenacoxib concentration was measured by liquid chromatography- tandem mass spectrometry (LC-MS/MS), with a 0.1-μg/m*l* lower limit of quantification. The analytical curve for robenacoxib concentration was determined by analysis of standard robenacoxib solutions by LC-MS/MS. The standard robenacoxib solution was prepared by dissolving 2.0 mg of reference standard robenacoxib (98% purity; lot no. L0513, Santa Cruz Biotechnology, Inc., Texas, U.S.A.) in 20 m*l* of 50% acetonitrile solution to obtain a 100-μg/m*l* solution of robenacoxib. This standard solution was serially diluted in 50% acetonitrile solution to obtain a series of standard solutions (0.1, 0.2, 0.5, 1.0, 2.0, and 5.0 μg/m*l*). The serum samples and the robenacoxib standard solutions (100 μl) were diluted in water (100 μl) and acetonitrile (200 μl) and centrifuged at 2,800 rpm for 10 min at 5°C. The supernatants were collected, suspended again in acetonitrile (50 μl in 100 μl), and centrifuged. The top clear layer obtained by centrifugation was used as the test sample for LC-MS/MS.

Liquid chromatography was performed using a high-throughput LC/MS system (1100 series, Agilent Technologies Japan, Ltd., Tokyo, Japan) and a reverse-phase column (Inertsil ODS-4; 3 μm; 2.1 × 150 mm; GL science Ltd., Tokyo, Japan). Column temperature was set at 40°C. The mobile phase comprised 0.1% formic acid and acetonitrile in a ratio of 1:19, and the test samples (3 μl) were delivered at a rate of 0.2 m*l*/min. Mass spectroscopic analysis was performed by tandem mass spectrometry (API3000, AB Sciex Ltd., Tokyo, Japan), using the negative electrospray mode for ionization (ion source temperature and voltage, 400°C and −3.0 kV, respectively). Ion transitions (Q1 mass ions were monitored at m/z 326.0; the declustering potential and collision energy were set at −66 V and −18 V, respectively.

Statistical analysis

The statistical analysis was performed using Statcel 3 (OMS, Tokyo, Japan). The data were reported as median [range: minimum-maximum] from 10 dogs. Pharmacokinetic properties such as a maximum serum concentration of robenacoxib (C_{max}; μg/m*l*), time of C_{max} (T_{max}; min), and serum robenacoxib concentration at 240 min (C_{240}) were compared between the dogs under the conscious condition and sevoflurane anesthesia using the Wilcoxon rank-sum test. For all analyses, values of $P<0.05$ were considered significant.

RESULTS

Sevoflurane MAC and cardio-respiratory measurements in anesthetized dogs

The sevoflurane MACs were 2.0% [range: 1.5–2.4] in 10 dogs. The dogs were anesthetized with 2.6% [range: 2.0–3.1] of ETsev to deliver the 1.3-fold of sevoflurane MAC. The cardio-respiratory measurements during the sevoflurane anesthesia were summarized in Table 1. No clinically apparent hypotension (MABP <60 mmHg) was observed although 6 dogs showed low arterial blood pressures (MABP 60–65 mmHg) during the sevoflurane anesthesia.

Changes in serum robenacoxib concentration

Figure 1 showed serum concentration-time profiles for robenacoxib in the dogs under the conscious condition or sevoflurane anesthesia. The C_{max}, T_{max}, and C_{240} in the conscious dogs or sevoflurane-anesthetized dogs were summarized in Table 2. In the sevoflurane-anesthetized dogs, C_{max} significantly declined (magnitude of difference between the conscious dogs and anesthetized dogs: 1.1 μg/m*l*, confidence interval [CI] 95%: 0.68–1.84 μg/m*l*, P=0.008) and T_{max} was delayed (magnitude of difference between the conscious dogs and anesthetized dogs: 120 min, CI 95%: 74–142 min, P=0.018) compared with those of the conscious dogs. The C_{240} decreased in the conscious dogs, while it remained higher in the sevoflurane-anesthetized dogs (P=0.011).

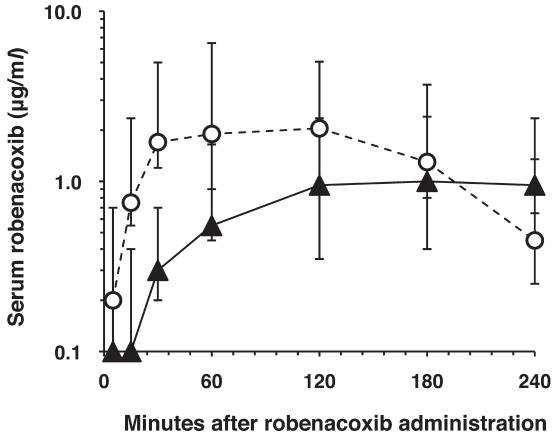

Fig. 1. Serum concentration–time profiles for robenacoxib in the conscious and sevoflurane-anesthetized dogs. Each plot and error bar represented median value and range (minimum-maximum) from 10 dogs under conscious condition (○) or sevoflurane anesthesia (▲).

Table 2. Maximum serum concentration of robenacoxib (C_{max}), time of C_{max} (T_{max}), and serum robenacoxib concentration at 240 min after the administraton (C_{240}) in dogs under consciousness or sevoflurane anesthesia

	Under consciousness	Under sevoflurane anesthesia
C_{max} (μg/m*l*)	2.2 [1.2–4.6]	1.3 [0.8–1.4] [b]
T_{max} (min)	90 [60–120]	120 [120–240] [a]
C_{240} (μg/m*l*)	0.5 [0.2–0.9]	1.0 [0.3–1.4] [a]

Data showed median [range]. Singnificant difference from the dogs under consciousness: a) $P<0.05$ and b) $P<0.01$.

DISCUSSION

The present study showed that the sevoflurane anesthesia affected the distribution of robenacoxib that was subcutaneously administered to healthy dogs. Significant decline in C_{max}, prolonged T_{max}, and higher remaining C_{240} of robenacoxib were observed in the dogs under the sevoflurane anesthesia. It was supposed that cardiovascular effects of sevoflurane might be responsible for the decline in the distribution of subcutaneously administered robenacoxib [1, 2, 18]. In any case, the robenacoxib C_{max} of the sevoflurane-anesthetized dogs should suffice to provide suppressive effect on pain and inflammation according to the result of previous reports in dogs with osteoarthritis [4, 23]. Therefore, the sevoflurane anesthesia may minimally disturb the anti-inflammatory and analgesic effects of robenacoxib in dogs.

Jung *et al.* [11] reported that the maximum blood concentration of robenacoxib after a subcutaneous administration of robenacoxib (1 mg/kg) was 657 *n*g/m*l* (nearly 0.7 μg/m*l*) at 30 min post-injection in healthy conscious dogs. In the present study, the C_{max} and T_{max} ranged from 1.2 to 4.6 μg/m*l* and from 60 to 90 min after a subcutaneous administration of robenacoxib (2 mg/kg) in the conscious dogs, respectively. Considering the differences in the dosage of robenacoxib and the concentration between blood and serum samples, the distribution of robenacoxib in the conscious dogs of the present study corresponded approximately with those in the previous study [11]. The changes in serum robenacoxib concentration in the conscious dogs similar to the previous report were reproduced in the present study.

Borer *et al.* [4] showed that the onset of analgesic action and time of peak effect of oral robenacoxib (2 mg/kg) were produced at 2–2.5 hr and 3–5 hr after the oral administration in conscious dogs with the urate crystal-induced acute stifle synovitis. In these dogs with acute stifle synovitis, the mean C_{max}, median T_{max}, and mean C_{240} of robenacoxib were 582.6 *n*g/m*l* (nearly 0.6 μg/m*l*), 1 hr, and lower than 0.1 μg/m*l*, respectively [4]. Compared to these dogs with acute stifle synovitis, the conscious dogs demonstrated higher C_{max} and C_{240} values in the present study. Jung *et al.* [11] reported that there were no significant differences in C_{max} values between subcutaneous (nearly 0.7 μg/m*l*) and oral (nearly 0.9 μg/m*l*) administrations of robenacoxib (1 mg/kg) in conscious healthy dogs. Owing to its acidic nature, robenacoxib is highly bound to plasma proteins with the result that it concentrates in inflamed tissues such as an arthritic joint [23], thereby displaying the property of tissue selectivity [5]. The higher serum concentrations of robenacoxib developed in the previous [11] and present study might be caused by the lack of inflamed tissue in the dogs. Although the blood concentration of robenacoxib may not have a linear relationship with the effect of this drug, the higher blood concentration of robenacoxib in healthy dogs may guarantee the compatibility to distribute and provide suppressive effect on pain and inflammation if the inflammations occur.

In the present study, significant decline in C_{max}, prolonged T_{max}, and higher remaining C_{240} of robenacoxib were caused by the sevoflurane anesthesia. It is well known that inhalation anesthetic not only reduce total blood flow (i.e., cardiac output) but they also impact the distribution of blood flow to tissues [25]. It was thought that the sevoflurane anesthesia might slow down the distribution of robenacoxib that was subcutaneously administered in the dogs. The drugs injected into subcutaneous tissue are absorbed from the injected subcutaneous site into the peripheral blood vessels and then transferred into the systemic circulation. It has been reported that the pharmacokinetics of intravenously administered indocyanine green, inulin, and antypyrine are affected due to altered cardiac output and regional blood flow distribution caused by isoflurane in dogs induced anesthesia with methohexital (10–15 mg/kg intravenously) [1]. In this previous study, cardiac output and MABP were decreased from 4.55 ± 1.13 *l*/min (mean ± standard deviation) and 102 ± 8 mmHg of baseline value in awake to 3.64 ± 0.36 *l*/min and 78 ± 6 mmHg under

the 1.15 MAC of isoflurane anesthesia in artificially ventilated dogs weighing 24–37 kg [1]. Sevoflurane causes cardio-respiratory depression in a dose-dependent manner in dogs similar to isoflurane [19, 20, 25]. For volatile anesthetics, the dosage required to maintain the surgical depth of anesthesia ranges from 1.2 to 1.4 MAC [25]. In the present study, the dogs were anesthetized with the 1.3 MAC of sevoflurane to mimic the surgical depth of anesthesia. Mutoh *et al.* [20] reported that a minimal change in cardiac output accompanied with a slight but significant decrease in arterial blood pressure and an increase in heart rate was produced in spontaneously breathing dogs anesthetized with 1.0 and 1.5 MAC of sevoflurane. Bernard *et al.* [3] also reported that 1.2 MAC of sevoflurane induced a minimal change in cardiac output accompanied with an increase in heart rate and decreases in mean aortic blood pressure (−22% compared to awake spontaneously breathing dogs) and portal blood flow (−14%) in mechanically ventilated dogs. In the present study, 6 dogs showed decreases in arterial blood pressure during the sevoflurane. It is thought that the 1.3 MAC of sevoflurane anesthesia might produce a minimal change in cardiac output but decrease regional blood flow distribution and result in delayed absorption and transfer of robenacoxib from the injected site to systemic circulation, which would explain the delay in detection of serum robenacoxib as well as the declined C_{max}.

COX-2 is induced by tissue damage to generate pro-inflammatory prostaglandins (PGs) such as PGE_2 and PGI_2 [17]. Therefore, COX-2 was classified as an inducible isoform, interacting with other mediators such as histamine and bradykinin to generate the cardinal signs of inflammation (heat, redness, swelling, pain and loss of function). Robenacoxib is a highly selective COX-2 inhibitor, developed for use in dogs and cats for the suppression of pain and inflammation [11, 13]. Whole blood *in vitro* assays are used to investigate quantitatively efficacy, potency, and sensitivity for NSAID inhibition of COX isoforms, providing data on COX-1: COX-2 inhibition ratios [17]. COX-1 activity is determined by measuring serum thromboxane B_2 synthesis in blood samples that allows blood to clot and COX-2 activity is determined by measuring prostaglandin PGE_2 synthesis in blood samples in the presence of lipopolysaccharide [14, 17, 23]. *In vivo*, a concentration producing 80% inhibition of COX-2 ($IC_{80, cox2}$) is typically required to elicit a clinical analgesic effect [17] and the $IC_{80, cox2}$ of robenacoxib is reported to be 77.01 *n*g/m*l* (approximately 0.08 μg/m*l*) in dogs with osteoarthritis [14, 23]. In the present study, the levels of robenacoxib C_{max} were higher than the $IC_{80, cox2}$ of robenacoxib in all of the conscious and sevoflurane-anesthetized dogs. Converting the $IC_{80, cox2}$ into serum robenacoxib concentration using normal value of packed cell volume for dogs (40–50%), the corresponding serum robenacoxib concentration to the $IC_{80, cox2}$ ranges from 0.16 to 0.2 μg/m*l*. The serum robenacoxib concentrations were over 0.2 μg/m*l* from 30 to 180 min after the administration in all of the conscious dogs. In addition, the serum robenacoxib concentrations over 0.2 μg/m*l* were also achieved from 120 to 240 min after the administration in all of the sevoflurane-anesthetized dogs. The level of robenacoxib C_{max} under the sevoflurane anesthesia was higher enough to provide anti-inflammatory and analgesic effects in dogs with osteoarthritis. Although there is no information about the effective blood concentration of robenacoxib to provide anti-inflammatory and analgesic effect on acute pain induced by surgery, the sevoflurane anesthesia may minimally disturb the anti-inflammatory and analgesic effect of robenacoxib in dogs.

A concentration producing 20% inhibition of the COX-1 isoform ($IC_{20, cox1}$) is a surrogate marker for safety of NSAIDs. Selectivity expressed as the clinically relevant ratio $IC_{20, cox1}$: $IC_{80, cox2}$ is highest for robenacoxib (19.8) compared to deracoxib (2.3), carprofen (2.5 and 2.1), nimesulide (1.8), etodolac (0.76), meloxicam (0.46), and ketoprofen (0.21) in dogs [14]. Therefore, the $IC_{20, cox1}$ of robenacoxib is 1,524.8 *n*g/m*l* (approximately 1.5 μg/m*l*) and corresponding serum robenacoxib concentration using normal value of packed cell volume for dogs (40–50%) ranges from 3.0 to 3.8 μg/m*l*. The C_{max} of robenacoxib did not reach the $IC_{20, cox1}$ except for a conscious dog of 1-year old (4.6 μg/m*l* of the C_{max} at 60 min) in the present study. So, sevoflurane anesthesia would not enhance adverse effects of robenacoxib in dogs whether it is subcutaneously administered at clinically recommended doses. However, it is necessary to be careful interpretation because there is variability of $IC_{20, cox1}$ among the experimental method.

Our study has three major limitations. First, we measured serum robenacoxib concentrations until 240 min but could not determine complete pharmacokinetic profiles of subcutaneously administered robenacoxib in the sevoflurane-anesthetized dogs. Jung *et al.* [11] showed that the blood concentration of robenacoxib peaked within 60 min and lowered until 240 min after the administration in conscious dogs. Based on this previous study, the blood samples were collected until 240 min after the administration of robenacoxib in the conscious and sevoflurane-anesthetized dogs. However, the plasma concentrations of robenacoxib did not decline at 240 min after the administration of robenacoxib in the most sevoflurane-anesthetized dogs. Therefore, the interpretation on the influence of sevoflurane anesthesia in the present study was limited on the distribution of subcutaneously administered robenacoxib in dogs. Second, clinically apparent cardiovascular depression was not demonstrated by heart rate and noninvasive arterial blood pressure measurements, although 6 dogs showed decreases in arterial blood pressure during the sevoflurane anesthesia. Sophisticated cardiovascular measurements including cardiac output and direct arterial blood pressure might be necessary to demonstrate the cardiovascular effect sevoflurane on the C_{max} and T_{max}. Third, the distribution of subcutaneously administered robenacoxib in the sevoflurane-anesthetized dogs might be affected by not only sevoflurane anesthesia but also mechanical ventilation and intravenously administered lactated Ringer's solution. Therefore, the results of the present study should be interpreted that the changes in the robenacoxib distribution of the sevoflurane-anesthetized dogs were caused by the combination of cardiovascular effects of sevoflurane anesthesia, mechanical ventilation, and fluid therapy. To clarify the effect of fluid loading on the distribution of robenacoxib, the group of conscious dogs received same dose of fluid should be added for proper discussion. Further studies are necessary to determine the exact effect of sevoflurane anesthesia on the pharmacokinetics of subcutaneously administered robenacoxib in dogs.

In conclusion, the distribution of subcutaneously administered robenacoxib was affected in the dogs anesthetized with sevoflurane, mechanically ventilated, and received fluid-therapy. However, these anesthetic procedures may minimally disturb the anti-inflammatory and analgesic effects of robenacoxib in dogs.

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