Master's Thesis of Veterinary Medicine

Effect of Bupivacaine and Temperature-responsive Hydrogel on Femoral and Sciatic Nerve Block in Beagle Dogs

Bupivacaine과 온도감응형 수화젤의 비글견 대퇴 및 좌골신경 차단 효과

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Abstract

This study was conducted to compare the duration of regional anesthesia of pelvic limb using bupivacaine with and without a temperature-responsive hydrogel (TRH) in dogs.

Under anesthesia using medetomidine (10 $\mu g \cdot kg^{-1}$), alfaxalone (2 $mg \cdot kg^{-1}$) and isoflurane, seven healthy male Beagle dog received four injections of 0.5% bupivacaine (1 $mg \cdot kg^{-1}$ with 5 $\mu g \cdot mL^{-1}$ epinephrine) to block femoral and sciatic nerves bilaterally via ultrasound with nerve stimulation guidance. Bupivacaine was used on one pelvic limb (Bup treatment), and bupivacaine with TRH was used on the contralateral limb (Bup-TRH treatment). The nerve block was considered successful upon the absence of responses to pinching the digital pads and mid-tibial skin of both pelvic limbs with mosquito forceps; the pinch, proprioception, and locomotion tests were performed before (baseline) and at each hour after the nerve block until sensory and motor functions returned to baseline. The effect of TRH on nerve blocks was analyzed using a linear mixed model.

The duration of the sensory nerve block at the digital pads and mid-tibial skin were longer with Bup-TRH (8.0 ± 1.6 and 10.9 ± 1.6 hours, respectively) than with Bup treatment (3.7 ± 2.0 and 8.0 ± 1.4 hours,

respectively). Motor block times of proprioception and locomotion were longer with Bup-TRH (9.3 ± 1.6 and 12.7 ± 1.5 hours, respectively) than with Bup treatment (4.6 ± 1.9 and 9.6 ± 1.5 hours, respectively). No complications were observed.

Therefore, TRH extended the duration of regional anesthesia of the pelvic limb using bupivacaine. TRH can be used for longer analgesia with local anesthetics in several nerve blocks.

Keyword: dog, hydrogel, nerve block, nerve stimulator, regional anesthesia, ultrasound-guided

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Introduction

Drug delivery systems for local anesthesia have been developed to extend the duration of a single injection of local anesthetic agents and to overcome the complications of multi-injection or constant-injection devices, including infection via a catheter-type device and required resource for long-term pain management, especially in animals that are unable to communicate and self-manage (Abelson *et al*. 2009). Drug delivery systems are typically categorized as injectable particles (nanoparticles and liposomes), injectable liquids (cyclodextrins, injectable liquid polymers, and hydrogels), and hybrid formulations (Santamaria *et al*. 2017). Nocita®, a form of liposome-encapsulated bupivacaine, is now a commercially available drug delivery system in veterinary medicine (Grubb & Lobprise 2020).

Temperature-responsive hydrogel (TRH) has recently been developed as a hyaluronic acid-based drug delivery system. Unlike other hydrogels with high viscosities that are challenging to inject and may require surgical implantation (Santamaria *et al*. 2017), TRH is an injectable, viscous fluid at 2–8 ℃ that becomes a gel at 37 ℃. The *in vitro* and *in vivo* biocompatibilities and preclinical feasibility tests of TRH are favorable, and minimal inflammatory reactions and no negative impacts on wound healing have been reported in rats (Kim *et al*. 2015; Oh *et al*. 2019; Seol *et al*. 2013). The use of TRH as an injectable and degradable hydrogel-based system in

the joint cavity also has a therapeutic effect on osteoarthritis in humans (Ayhan *et al*. 2014). The effect durations of bupivacaine and ropivacaine with TRH have been reported as 24–72 hours when administered subcutaneously or intra-articularly in rats and humans (Choi *et al*. 2019; Kim *et al*. 2015; Oh *et al*. 2019). For regional anesthesia, a rat study comparing the duration of sciatic nerve blocks using 0.5% bupivacaine with and without another hyaluronic acid-based drug delivery system reported that the effect duration almost doubled from 135 minutes to about 262 minutes, a 94% increase (Jia *et al*. 2004, Weiniger *et al*. 2010).

To evaluate the use of TRH for regional anesthesia in dogs, this study compared the duration of regional anesthesia of the pelvic limb using 0.5% bupivacaine to that when using 0.5% bupivacaine with a TRH. After performing femoral and sciatic nerve blocks using these agents, the sensory blockade of the digits and mid-tibial skin and the motor blockade (proprioception and locomotion) were compared in Beagle dogs. The hypothesis was that TRH would prolong the effects of regional anesthesia in the pelvic limb compared to bupivacaine alone.

Materials and methods

1. Animals

This prospective, blinded, randomized study was approved by the Institute Animal Care and Use Committee of the Seoul National University (SNU-200728-4). Seven healthy male Beagles with a mean weight of 8.8 ± 1.3 kg and a mean age of 14.3 ± 3.9 months were included in this study. They were obtained from a previous study unrelated to the pelvic limb. No abnormalities were found on physical examination, thoracic radiographs, complete blood cell count, and serum chemistry. All dogs were fasted for 12 hours prior to the study period and were given free access to water.

2. Anesthetic procedure

The dogs were sedated with intramuscular injections of medetomidine (10 $\mu g \cdot kg^{-1}$; Tomidine, Provet Veterinary Products Ltd., Istanbul, Turkey). An intravenous (IV) catheter (Sewoon Medical Co., Seoul, Republic of Korea) was placed in the cephalic vein, and Hartmann solution (JW Pharmaceutical, Seoul, Korea) was administered IV (5 $mL \cdot kg^{-1}$ $hour^{-1}$). The dogs were induced with IV alfaxalone (2 $mg \cdot kg^{-1}$; Alfaxan, Jurox, Australia) and intubated. The spontaneously breathing dogs were anesthetized using a rebreathing circuit with isoflurane (I-Fran Liquid, Hana Pharm Co., Ltd., Hwaseong, Republic of Korea) in 100% oxygen (1.5 $L \cdot minute^{-1}$) at a target of 1.5% end-tidal isoflurane concentration (F_E'Iso).

The dorsal pedal artery was catheterized, and the catheter was connected to a transducer system (TruWave; Edwards Lifesciences, Germany) filled with heparinized saline. The transducer was placed at the level of the right atrium and zeroed to atmospheric pressure. The heart rate (HR), arterial oxygen saturation (SpO_2), systolic arterial pressure (SAP), mean arterial pressure (MAP), diastolic arterial pressure (DAP), respiratory rate (f_R), end-tidal partial pressure of carbon dioxide ($P_E'CO_2$) from capnography, esophageal temperature (T), and F_E'Iso were monitored continuously with a multiparameter monitor (Datex-Ohmeda AS/3; GE Healthcare, Finland) and recorded every 5 minutes. After all procedures, atipamezole (50 $\mu g \cdot kg^{-1}$; Reversal, Provet Veterinary Products Ltd., Istanbul, Turkey) was used to reverse the sedation.

3. Preparation of local anesthetic solution

The 0.5% bupivacaine (Bupivacaine HCl, Myeongmoon Pharm. Co., Hwaseong, Republic of Korea) used in this study included epinephrine (5 $\mu g \cdot mL^{-1}$; Epinephrine Injection, Dai Han Pharm. Co., Ansan, Republic of Korea). The TRH (PF-72, TGel Bio, Co., Ltd., Korea), a lyophilized powder, was mixed with 10 mL of 0.5% bupivacaine and refrigerated at 2–8 ℃ for one day prior to use (Kim *et al*. 2015).

4. Regional anesthesia for the pelvic limb

The dogs were positioned in lateral recumbency. The bilateral skin from the sacrum to the mid-femur and inguinal region were clipped and prepared aseptically. Each dog underwent femoral and sciatic nerve blocks using 1 $mg \cdot kg^{-1}$ (0.2 $mL \cdot kg^{-1}$ per nerve) of 0.5% bupivacaine in the right or left pelvic limb (Bup treatment) and femoral and sciatic nerve blocks using 1 $mg \cdot kg^{-1}$ of 0.5% bupivacaine mixed with TRH in the contralateral pelvic limb (Bup-TRH treatment). The total injected dose of bupivacaine was 4 $mg \cdot kg^{-1}$ in each dog. The pelvic limb, nerve block order, and drug types were randomized via drawing lots. All nerve blocks were performed by one experienced investigator who is skilled in ultrasound-guided nerve blocks. The ultrasound-guided perineural femoral and sciatic nerve blocks were performed as follows: the femoral nerve was blocked in the iliopsoas muscle before its division into the sartorius and quadriceps femoris muscles using a ventral suprainguinal approach and the inguinal nipple as a landmark **(**Echeverry *et al*. 2012). The sciatic nerve was blocked at the area immediately distal to the greater trochanter and the ischiatic tuberosity (Campoy *et al*. 2010). Ultrasonographic equipment (Samsung Medison H60, Samsung Medison Co., Ltd., Seoul, Korea) with a linear transducer was used for the visualization of the needle with an in-plane technique for the approach. An echogenic insulated needle with a short bevel (20 gauge, 150 mm, Sonoplex Nanoline Stim Cannula, Pajunk, Geislingen, Germany) was inserted in the long axis of the ultrasound transducer and advanced toward

the target nerve. A nerve stimulator (EZstim II ES400, Life-Tech, Stafford, TX, USA), set at a current of 0.3 mA, a frequency of 2 Hz, and a pulse duration of 0.1 ms, was connected to the insulated needle and confirmed the proximity of the needle tip to the target nerve. To avoid an IV injection, a negative aspiration test was performed before injection. The longitudinal distribution of the local anesthetic agent along the femoral or sciatic nerve was observed using transverse ultrasound images while moving the transducer along the nerves. The same method was used for both injections.

5. Evaluation and data collection

A two-point rating scale (the presence/absence of sensory response and motor function deficit) was used for all assessments. For the sensory blockade assessment, the dogs were placed in a natural standing position and gently restrained by an investigator standing in front of and distracting the dogs. Another investigator who was blinded to the treatment of each limb stood behind the dog being assessed and pinched the digital pads and mid-tibial skin of the pelvic limbs using mosquito forceps (H112-22012; Hermann Medizintechnik, Germany) when the dog looked forward so that the pinching could not be seen by the dog. The presence of responses to pinching the digits and mid-tibial skin was ascertained by the dog turning its head, actively looking at the stimulated area, and/or vocalization. Complete nerve block (i.e. both limbs) was considered to be successful if there were no responses to pinching all four digital pads and all four points of mid-

tibial skin in both pelvic limbs and if there was loss of proprioception and locomotion for at least one hour following administration of the nerve blocks. If no reactions were observed, the first ratchet was closed for one second for equal pressure and released. If a baseline sensory response was noted before closing the ratchet, the ratchet was released immediately. The tests were repeated each hour after the nerve block, and they were stopped when pain responses were observed. The duration of the sensory block was defined as the time from the nerve block to the last timepoint with no sensory responses in pinching points.

The motor blockade was assessed via tests for proprioception and locomotion for each limb. Proprioception was considered as the immediate replacement of the digit on its plantar surface when the dorsal surface was placed on the ground, and it was confirmed to be normal bilaterally before the nerve blocks. Delayed or no reposition of the limb was considered a negative result. Locomotion was evaluated in terms of weight bearing on the limb and the ability to walk in a straight line. Control and assessment locomotion tests were recorded on video (iPhone 8 and 11; Apple, CA, USA) for 2–3 minutes of walking. Full weight bearing on the limb and the ability to walk without missteps was considered as the baseline. Partial or no weight bearing on the limb or the presence of missteps was considered to be the presence of a motor-function deficit. The duration of the motor block was defined as the time from the nerve block to the last timepoint with a motor-function deficit.

Prior to anesthesia, control pinching responses, normal proprioception, and normal locomotion were measured. After full recovery from anesthesia, the tests were repeated every hour until all control values of all categories had returned. Between evaluations, the dogs rested in a cage to prevent skin damage caused by dragging of the legs. Meloxicam (0.2 $mg \cdot kg^{-1}$; Metacam, Boehringer Ingelheim, Barcelona, Spain) was administered subcutaneously after the study period.

6. Statistical analyses

All statistical analyses were performed using SPSS Statistics for Windows, Version 26 (IBM Corporation, NY, USA). A linear mixed model was used with pinching levels, pinching points and type of drug solutions to assess the influence of different variables on the sensory blockade duration of bupivacaine. The analysis of the duration of the motor blockade (proprioception and locomotion) was performed using a linear mixed model. Akaike's information criterion was used to compare different variance-covariance structures for the mixed model. A quantile-quantile plot was used to assess the distribution of the residuals. A type III analysis of variance was performed with a fixed model. Bonferroni correction was performed for *post hoc* comparisons. The pinching point and type of drug measurements were presented as the mean ± standard deviation. A value of $p \leq 0.05$ was considered statistically significant.

Results

1. Physiological data during anesthesia

Under general anesthesia, physiological values were as follows: the HRs of the dogs 50–120 beats per minute, SpO_2 95–99%, SAP 92–167 mmHg, MAP 62–106 mmHg, DAP 51–91 mmHg, f_R 8–25 breaths per minute, $P_E'CO_2$33–45 mmHg, and T 36.5–38 ℃.

2. Nerve block procedures

On the ultrasound images, the doughnut sign, indicating the distribution of local anesthetic around the nerve, was observed at the injection level in all dogs. Non-circumferential distribution was observed as the anesthetic spread from the injection site. The measured spreading distances of the local anesthetic agent along the nerve were 2.5–4.0 cm around the femoral nerve and 2.3–6.0 cm around the sciatic nerve for the Bup treatment and 2.0–4.0 cm around the femoral nerve and 2.5–7.0 cm around the sciatic nerve for the Bup-TRH treatment.

3. Duration of nerve blocks

The overall duration of complete sensory blockade at the digital pad and mid-tibial skin was longer ($p \leq 0.001$) with the Bup-TRH treatment (8.0 ± 1.6 and 10.9 ± 1.6 hours, respectively) than with the Bup treatment (3.7 ± 2.0 and 8.0 ± 1.4 hours, respectively). The duration of the sensory

blockade was significantly different between the four points at each level (p = 0.011) (Table 1). The recovery times of proprioception ($p \leq 0.001$) and locomotion (p = 0.002) were also longer with the Bup-TRH treatment (9.3 ± 1.6 and 12.7 ± 1.5 hours, respectively) than with the Bup treatment (4.6 ± 1.9 and 9.6 ± 1.5 hours, respectively) (Table 2).

4. Complications

No signs of cardiovascular or central nervous system toxicities related to the local anesthetic agents, such as hypotension, seizure, respiratory depression, or coma, were observed. No abnormalities in behavior or gait were observed a day and a week after the procedure.

Table 1. Complete sensory blockade duration of femoral and sciatic nerve blocks with bupivacaine (0.5% with 5 $\mu g \cdot mL^{-1}$ epinephrine) in the right or left pelvic limb (Bup treatment) and bupivacaine (0.5% with 5 $\mu g \cdot mL^{-1}$ epinephrine) with temperature-responsive hydrogel in the contralateral pelvic limb (Bup-TRH treatment).

Level	**Point**	**Duration (hours)**	
		Bup treatment	**Bup-TRH treatment**
Digital pad	Second	3.7 ± 2.1	8.1 ± 1.9
	Third	3.7 ± 2.1	8.0 ± 1.6
	Fourth	3.7 ± 2.1	8.0 ± 1.6
	Fifth	3.7 ± 2.1	8.0 ± 1.6
	Overall	3.7 ± 2.0	8.0 ± 1.6
Mid-tibia	Medial	8.9 ± 1.2	11.1 ± 1.6
	Lateral	7.9 ± 1.6	10.7 ± 1.8
	Cranial	7.7 ± 1.4	11.0 ± 1.6
	Caudal	7.7 ± 1.5	10.7 ± 1.8
	Overall	8.0 ± 1.4	10.9 ± 1.6
Linear mixed model	Treatment	$p \leq 0.001$	
	Level	$p = 0.001$	
	Point	$p = 0.011$	

Data are presented as the mean ± standard deviation

Table 2. Complete motor blockade duration of femoral and sciatic nerve blocks with bupivacaine (0.5% with 5 $\mu g \cdot mL^{-1}$ epinephrine) in the right or left pelvic limb (Bup treatment) and bupivacaine (0.5% with 5 $\mu g \cdot mL^{-1}$ epinephrine) with temperature-responsive hydrogel in the contralateral pelvic limb (Bup-TRH treatment).

Level	**Duration (hours)**	**Duration (hours)**	***p***
	Bup treatment	**Bup treatment**	
Proprioception	4.6 ± 1.9	9.3 ± 1.6	$p \leq 0.001$
Locomotion	9.6 ± 1.5	12.7 ± 1.5	$p = 0.002$

Data are presented as the mean ± standard deviation

Discussion

TRH significantly prolonged the sensory and motor nerve block effects in regional anesthesia of the pelvic limb. The results support the hypothesis that TRH would last longer the duration of the digital and mid-tibial skin sensory blockade and the proprioception deficits caused by regional anesthesia of the pelvic limb using 0.5% bupivacaine. Significant differences were found in the sensory nerve block durations at the four points of each level. These differences may be due to the different durations of the femoral and sciatic nerve blocks, as the femoral nerve is mainly responsible for the sensory innervation of the medial aspect of the pelvic limb while the sciatic nerve is responsible for the lateral aspect (Evans & De Lahunta 2013). The relatively longer duration of the femoral nerve block is similar to findings of previous studies that used bupivacaine for femoral and sciatic blocks in dogs (O Cathasaigh *et al*. 2018; Portela *et al*. 2010).

The evaluation of TRH as a drug delivery system requires proper methods to perform a reproducible nerve block and to objectively assess pain. In most dogs, femoral and sciatic nerve blocks are achieved using reliable anatomical landmarks that are consistently reproducible (Campoy & Mahler 2013). The use of ultrasound and nerve stimulator guidance improves the success rate of these procedures. The nerve block procedures used in this study both have a low learning curve, low anatomical risks, and a high success rate for complete motor and sensory blocks in dogs (Campoy

et al. 2010; Echeverry *et al*. 2012; O Cathasaigh *et al*. 2018). Both femoral and sciatic nerve blocks were necessary for regional anesthesia of the pelvic limb and facilitated a complete motor blockade (Marolf *et al*. 2019).

Considering the objective of the drug delivery system, the extended durations of complete sensory blockade at the digital pad and mid-tibial skin when using TRH (8.0 ± 1.6 and 10.9 ± 1.6 hours, respectively) may be considered insufficient compared to previous reports. A human study reported that ropivacaine with TRH administered directly to the surgical sites relieved pain for up to 72 hours based on patient self-reported pain scores (Choi *et al*. 2019). An *in vivo* rat study demonstrated significant pain relief for at least two days based on the weight-load and paw-withdrawal thresholds when bupivacaine with TRH was injected into the joint cavity (Kim *et al*. 2015). In addition, TRH with ropivacaine injected subcutaneously in the area of the sciatic nerve in rats resulted in pain relief for 24 hours based on the paw-withdrawal threshold (Oh *et al*. 2019). However, the blockade duration may be evaluated differently according to patient species, the size of related nerves, relative dose administered to animals and sites, and the evaluating methods including the difference in intensity of measurement; furthermore, the use of a two-point rating scale for excluding subjective or biased assessment in nonverbal animals may result in an underestimation of the analgesic duration in the present study. The sensory duration of a partial block is approximately 1.5–2 times longer than that of a complete block, as reported in studies using bupivacaine or

ropivacaine to achieve femoral and sciatic nerve blocks in dogs (Portela *et al*. 2010; Trein *et al*. 2017). Therefore, when TRH is used with local anesthetic agents for post-operative pain relief, the duration of pain relief, including complete and partial nerve blocks, would be longer than reported by the current study. Further studies that include both partial and complete blockades are needed to determine the period of significant pain relief with TRH in a clinical setting.

The volume and concentration of local anesthetic agents are important determinants of the success and duration of complete peripheral nerve blocks (Portela *et al*. 2010), and a high success rate was needed for complete block of all four nerves in the present study. A previous study reported that 0.25% of bupivacaine was inadequate for complete femoral and sciatic nerve blocks and that complete nerve blocks were achieved with 0.15 $mL \cdot kg^{-1}$ of 0.5% bupivacaine (O Cathasaigh *et al*. 2018). Other studies demonstrated that 0.2 $mL \cdot kg^{-1}$ of 0.5% bupivacaine was adequate for sensory blockade of the sciatic nerve even with ultrasound guidance and it increases the success rate, minimize variable outcomes, and is reproducible (Futema *et al*. 2002; Marolf *et al*. 2019). In a preliminary experiment, various concentrations and volumes were tested to achieve complete pelvic-limb blocks. While 0.1 $mL \cdot kg^{-1}$ of 0.25%, 0.1 $mL \cdot kg^{-1}$ of 0.5%, and 0.2 $mL \cdot kg^{-1}$ of 0.25% bupivacaine were noted to have a low possibility of a complete block, 0.2 $mL \cdot kg^{-1}$ of 0.5% bupivacaine resulted in a complete block with a relatively high success rate when the doughnut sign was

observed, similar to previous studies. This may be due to the circumferential spread of the local anesthetic agent around the sciatic nerve, and the higher volume of anesthetic agent may lead to more consistent reproducibility (Shilo *et al*. 2010). In addition, the local anesthetic agent in contact with > 2 cm of the nerve indicates a clinically effective peripheral nerve block because the length of the nerve in contact with the local anesthetic agent determines the success of the nerve block (Campoy *et al*. 2008). Although measuring the whole distances of local anesthetic agents around the target nerves were limited by bone shadows on the ultrasound images as moving the transducer upward, the local anesthetic agent in contact with > 2 cm of the nerve was observable in the present study when using 0.2 mL·kg^{-1} of the local anesthetic agent. The used volumes and concentrations of bupivacaine in this study were chosen to achieve the conditions of complete blockade, which was a requirement for data collection. The results indicate that the concentration and volume of local anesthetic agents used were adequate for sensory and motor blockade.

In this study, the total dose of bupivacaine used per dog was 4 mg·kg^{-1}, which exceeded the clinical dose for cardiotoxicity (2 mg·kg^{-1}). To compare the duration of the effect of a local anesthetic agent by two different treatments on the same dog at the same time, both femoral and sciatic nerve blocks were required in the pelvic limbs. Since the dose of bupivacaine per nerve was fixed as mentioned above, the total dose of bupivacaine used per individual increased. If a clinical application is

performed based on the findings herein, the total dose of bupivacaine per dog would not exceed the toxic dose because in a clinical environment, surgery is typically performed on one pelvic limb. Although the blood concentration of the local anesthetic was not measured here, the estimated blood concentration resulting from a 4 $mg \cdot kg^{-1}$ bupivacaine injection was estimated to be 4.16 $\mu g \cdot mL^{-1}$ based on a previous study using bupivacaine for femoral nerve blocks in dogs; the peak plasma concentration of bupivacaine was 0.78 $\mu g \cdot mL^{-1}$ when administering 0.75 $mg \cdot kg^{-1}$ of bupivacaine for femoral nerve blocks in dogs without epinephrine (O Cathasaigh *et al*. 2018). This concentration is lower than that reported to cause cardiovascular collapse in dogs (5.7 $\mu g \cdot mL^{-1}$) (Groban *et al*. 2001). Furthermore, most known convulsing and cardiovascular toxic doses are related to IV injection, but even relatively high plasma concentrations caused by large doses do not usually cause systemic toxicity if a significant dose of local anesthetic is not deposited intravascularly (Rosenberg *et al*. 2004). The addition of 5 $\mu g \cdot mL^{-1}$ epinephrine (1:200,000) into the local anesthetic agent reduces its peak plasma concentration without prolonging the duration of its effect (Rosenberg *et al*. 2004). Although the peripheral application of epinephrine may worsen nerve injury due to physical nerve damage or local anesthetic neurotoxicity in animals, the risk of nerve damage from peripheral block is extremely low in normal conditions (Neal 2003). Moreover, TRH delays the release of the local anesthetic agent, resulting in 60% lower blood concentration of the local anesthetic agent and

significantly lower concentration in various tissues compared to the free-form local anesthetic agent in rats (Oh *et al*. 2019). Similarly, liposome-encapsulated bupivacaine, a sustained-release drug delivery system, has a significantly higher tolerated dose than bupivacaine for inducing seizures and ventricular tachycardia in rabbits (Boogaerts *et al*. 1993). Epidural administration of lidocaine with hyaluronic acid, the ingredient of TRH, reduced the mean plasma lidocaine concentration by 50% compared to the administration of plain lidocaine in dogs (Doherty *et al*. 1996). The perineural injection site, addition of epinephrine, and characteristics of TRH suggest that the plasma concentration of bupivacaine may be lower than the toxic dose in dogs even with the administration of 4 $mg \cdot kg^{-1}$ of bupivacaine, although further studies regarding the application of epinephrine in TRH are needed. Fortunately, despite the high dose of bupivacaine used in this experiment for distinct objective evaluation, no related complications were observed. Nonetheless, a lower dose of bupivacaine should be used for further clinical trials and applications as a high dose of bupivacaine may lead to critical complications.

TRH, which offsets the disadvantages of highly viscous hydrogels, is still relatively viscous and requires increased injection pressure. Although this may be compensated with the use of a large-gauge needle with a short length, an intraneural injection cannot be ruled out owing to the injection pressure. Ultrasound guidance is necessary to prevent an intraneural injection and was used in this study. The requirement of increased injection

pressure prevented the blinding of the anesthetist who performed the nerve blocks. However, the pinching test and motor-function assessments were conducted by an investigator who was blinded to the treatment.

This study was confined to regional anesthesia in Beagle dogs; thus, more clinical studies regarding the duration of significant pain relief in various dog breeds and other types of regional anesthesia are necessary. Although the objectivity of the assessment has been ensured as much as possible, there is still a limitation of only a single investigator having assessed the dogs. In addition, the instability in the limb that recovered slower affected the weight bearing and walking abilities of the opposite limb and resulted in the overestimation of the locomotor function of the limb that recovered faster.

Conclusions

TRH extended the duration of regional anesthesia of the pelvic limb achieved using bupivacaine for digital sensory blockade from 3.7 ± 2.0 to 8.0 ± 1.6 hours, for mid-tibial skin sensory blockade from 8.0 ± 1.4 to 10.9 ± 1.6 hours, and for proprioception from 4.6 ± 1.9 to 9.3 ± 1.6 hours. TRH is an applicable drug delivery system for extending the duration of regional anesthesia of the pelvic limb using bupivacaine in dogs.

References

Abelson AL, McCobb EC, Shaw S *et al*. (2009) Use of wound soaker catheters for the administration of local anesthetic for post-operative analgesia: 56 cases. Vet Anaesth Analg 36, 597-602.

Ayhan E, Kesmezacar H, Akgun I (2014) Intraarticular injections (corticosteroid, hyaluronic acid, platelet rich plasma) for the knee osteoarthritis. World J Orthop 5, 351-361.

Boogaerts J, Declercq A, Lafont N *et al*. (1993) Toxicity of bupivacaine encapsulated into liposomes and injected intravenously: comparison with plain solutions. Anesth Analg 76, 553-555.

Campoy L, Martin-Flores M, Looney AL *et al*. (2008) Distribution of a lidocaine-methylene blue solution staining in brachial plexus, lumbar plexus and sciatic nerve blocks in the dog. Vet Anaesth Analg 35, 348-354.

Campoy L, Bezuidenhout AJ, Gleed RD *et al*. (2010) Ultrasound-guided approach for axillary brachial plexus, femoral nerve, and sciatic nerve blocks in dogs. Vet Anaesth Analg 37, 144-153.

Campoy L, Mahler S (2013) The Pelvic Limb. In: Small Animal Regional Anesthesia and Analgesia (1st edn). Campoy L, Read MR (eds). Wiley-Blackwell, USA, pp. 199-226.

Choi SW, Jang HD, Kang DW *et al*. (2019) Comparison of the efficacy and safety profiles of a mixed ‘PF-72’ and ‘0.75% ropivacaine HCl’

versus a ‘0.75% ropivacaine HCl’ and no treatment group: A randomized, single-blind, single-institution pilot study. J Korean Soc Spine Surg 26, 11-20.

Doherty MM, Hughes PJ, Charman SA, Brock KV, Korszniak NV, Charman WN (1996) Biphasic drug absorption from the epidural space of the dog may limit the utility of a slow release medium molecular weight hyaluronic acid- lidocaine ionic complex formulation. Anesth Analg. 83, 1244-1250.

Echeverry DF, Laredo FG, Gil F *et al*. (2012) Ventral ultrasound-guided suprainguinal approach to block the femoral nerve in the dog. Vet J 192, 333-337.

Evans HE, De Lahunta A (2013) Spinal nerves. In: Miller’s Anatomy of the Dog (4th edn). Evans HE, De Lahunta A (eds). Elsevier Health Sciences, USA, pp. 611-656.

Futema F, Fantoni DT, Auler JOC Jr. *et al*. (2002) A new brachial plexus block technique in dogs. Vet Anaesth Analg 29, 133-139.

Groban L, Deal DD, Vernon JC *et al*. (2001) Cardiac resuscitation after incremental overdosage with lidocaine, bupivacaine, levobupivacaine, and ropivacaine in anesthetized dogs. Anesth Analg 92, 37-43.

Grubb T, Lobprise H (2020) Local and regional anaesthesia in dogs and cats: Overview of concepts and drugs (Part 1). Vet Med Sci 6, 209-217.

Jia X, Colombo G, Padera R *et al*. (2004) Prolongation of sciatic nerve blockade by in situ cross-linked hyaluronic acid. Biomaterials 25, 4797-4804.

Kim T, Seol DR, Hahm SC *et al*. (2015) Analgesic effect of intra-articular injection of temperature-responsive hydrogel containing bupivacaine on osteoarthritic pain in rats. Biomed Res Int 2015, 812949.

Marolf V, Rohrbach H, Bolen G *et al.* (2019) Sciatic nerve block in dogs: description and evaluation of a modified ultrasound-guided parasacral approach. Vet Anaesth Analg 46, 106-115.

Neal JM (2003) Effects of epinephrine in local anesthetics on the central and peripheral nervous systems: neurotoxicity and neural blood flow. Reg Anesth Pain Med 28, 124-134.

O Cathasaigh M, Read MR, Atilla A *et al.* (2018) Blood concentration of bupivacaine and duration of sensory and motor block following ultrasound-guided femoral and sciatic nerve blocks in dogs. PLOS ONE 13, e0193400.

Oh KS, Hwang C, Lee HY *et al*. (2019) Preclinical studies of ropivacaine extended-release from a temperature responsive hydrogel for prolonged relief of pain at the surgical wound. Int J Pharm 558, 225-230.

Portela DA, Otero PE, Tarragona L *et al*. (2010) Combined paravertebral plexus block and parasacral sciatic block in healthy dogs. Vet Anaesth Analg 37, 531-541.

Rosenberg PH, Veering BT, Urmey WF (2004) Maximum recommended doses of local anesthetics: a multifactorial concept. Reg Anesth Pain Med 29, 564-575.

Santamaria CM, Woodruff A, Yang R, Kohane DS (2017) Drug delivery systems for prolonged duration local anesthesia. Mater Today (Kidlington) 20, 22-31.

Seol D, Magnetta MJ, Ramakrishnan PS *et al.* (2013) Biocompatibility and preclinical feasibility tests of a temperature-sensitive hydrogel for the purpose of surgical wound pain control and cartilage repair. J Biomed Mater Res B Appl Biomater 101, 1508-1515.

Shilo Y, Pascoe PJ, Cissell D *et al*. (2010) Ultrasound-guided nerve blocks of the pelvic limb in dogs. Vet Anaesth Analg 37, 460-470.

Trein TA, Floriano BP, Wagatsuma JT *et al*. (2017) Effects of dexmedetomidine combined with ropivacaine on sciatic and femoral nerve blockade in dogs. Vet Anaesth Analg 44, 144-153.

Weiniger CF, Golovanevski M, Sokolsky-Papkov M, Domb AJ (2010) Review of prolonged local anesthetic action. Expert Opin Drug Deliv 7, 737-752.

국 문 초 록

Bupivacaine과 온도감응형 수화젤의 비글견 대퇴 및 좌골신경 차단 효과

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본 연구는 개에서 bupivacaine과 혼합한 온도감응형 수화젤(temperature-responsive hydrogel; TRH)을 뒷다리 부위마취에 적용하였을 때 지속시간을 효과적으로 연장할 수 있는지를 알아보기 위하여 실시하였다.

Medetomidine 10 μg·kg^{-1}, alfaxalone 2 mg·kg^{-1}과 isoflurane을 이용해 전신마취 된 7 마리의 건강한 수컷 비글 견에서 5 μg·mL^{-1}의 epinephrine이 포함되어 있는 0.5% bupivacaine 1 mg·kg^{-1}을 초음파와 신경근 자극기를 이용하여 뒷다리 양쪽의 대퇴신경과 좌골신경을 차단하기 위해 4번 주사하였다. Bupivacaine은 한쪽 뒷다리에서 사용되었으며 Bup 투여군으로 지칭하였고, bupivacaine과 온도감응형 수화젤의 혼합물은 반대쪽 다리에서 사용되었으며 Bup-TRH 투여군으로 지칭하였다. 비글 견의 마취 회복 후 mosquitoes 지혈 겸자를 이용하여 양쪽 뒷다리의 발가락, 경골 수준의 피부를 자극하여 그에 대한 반응이

없는 것을 신경 차단의 성공으로 판단하였다. 국소 마취 전 피부 자극, 고유감각, 보행능력의 검사가 실시되었고, 국소 마취 이후 모든 항목이 기준선으로 돌아오는 시점까지 한 시간 간격으로 검사를 실시하였다. 온도감응형 수화젤의 효과는 선형혼합모델을 이용하여 분석되었다.

발가락과 경골 수준에서의 감각 차단 시간은 각각 3.7 ± 2.0과 8.0 ± 1.4 시간을 나타낸 Bup 투여군에 비해 각각 8.0 ± 1.6과 10.9 ± 1.6 시간을 나타낸 Bup-TRH 투여군에서 연장이 확인되었으며, 고유감각과 보행능력의 운동기능 차단 시간도 각각 4.6 ± 1.9과 9.6 ± 1.5 시간을 나타낸 Bup 투여군에 비해 각각 9.3 ± 1.6과 12.7 ± 1.5 시간을 나타낸 Bup-TRH 투여군에서 연장이 확인되었다.

본 연구 결과를 통하여, 온도감응형 수화젤의 사용은 bupivacaine을 이용해 실시하는 뒷다리의 국소마취의 지속시간을 유의미하게 연장하는 이점이 있음을 확인하였다. 온도감응형 수화젤을 이용하여 여러가지 부위마취에서 국소마취제의 진통시간을 연장할 수 있을 것으로 판단된다.

주요어: 개, 수화젤, 국소마취, 신경차단, 신경근 자극기, 초음파 가이드

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