Paravertebral brachial plexus block in sheep: a cadaveric and in vivo study

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Abstract

The Objective of the present study was to evaluate paravertebral brachial plexus block in sheep. A group of 13 healthy sheep with 20.10 ± 2.20 kg weight and five months of age were used. In phase I, in five sheep, an insulated needle attached to a nerve stimulator was directed to the location of C6, C7, C8 and T1 nerves and a 1.50 mL of a solution containing 1:1 methylene blue 1.00% and lidocaine 1.00% was injected at each site. Then, the cervical and thoracic areas were dissected and assessed in the cadavers. In phase II, cervical paravertebral block with 2.00% lidocaine and subsequent assessments were done in eight live sheep. Cadaveric evaluations revealed dye spread in C6 to T1 nerves: 61.75 ± 5.50, 72.75 ± 9.18, 40.75 ± 2.99 and 18.75 ± 3.30 mm, respectively. In three sheep, dye distribution in the anterior mediastinum was observed. In phase II, the onset of anesthesia were determined within 10 and 15 min for sensory and motor blocks, respectively. Anesthesia at axillary, musculocutaneous, radial and ulnar skin sites and motor block lasted for 67.50 ± 15.80, 63.70 ± 16.00, 55.00 ± 21.70, 56.70 ± 19.70 and 76.40 ± 24.30 min, respectively. In three sheep, no anesthesia was observed for radial and ulnar skin sites. In conclusion, paravertebral brachial plexus block in sheep provided an acceptable block for the upper parts of the elbow joint, however, it was not effective and reliable for more distal structures.

Introduction

In ruminants, most surgical procedures can be performed via a combination of physical restraint and regional or local anesthesia.1 The techniques of regional/local anesthesia are simple and do not commonly require special equipment. In addition, these techniques may be used in combination with general anesthesia aiming to diminish the doses of general anesthetics and providing more stable cardiorespiratory status.2

Brachial plexus block (BPB) is a regional anesthesia technique employed to provide analgesia in the thoracic limb. The nerves comprising of brachial plexus can be desensitized at different levels including at the site of emergence of spinal nerves in the cervical region (paravertebral approach; PBPB),3 at shoulder area into the axillary space (axillary approach; ABPB),4,7 and at mid-humeral region including radial, ulnar, median and musculocutaneous (RU/MM) block.8 In general, the higher the approach is, the more extensive the block shall be achieved.

Brachial plexus block is mostly performed in animals via axillary approach (i.e., ABPB). Although this technique is relatively easy to perform, several drawbacks have been reported including including the requirement of a large volume of solutions to be injected, slow onset time, absence of anesthesia for proximal structures of the elbow joint and the frequent occurrence of incomplete blocks.3 It seems that paravertebral approach (i.e., PBPB) has several advantages over conventional method. It has been suggested that, in PBPB, anatomic landmarks are more easily identified, smaller volumes of local anesthetic solution are injected, the entire limb is blocked and the block is more successful.3

In ruminates, axillary technique has also been employed as the prominent approach for BPB in several investigations.6,7,9–11 Recently, paravertebral approach as part of balanced anesthesia has been adopted for BPB in a lamb.12 To the authors’ knowledge, no experimental study has yet evaluated BPB in ruminants. Therefore, the present investigation was designed to assess the possibility and...
potential complications of performing PBPB in sheep using a nerve stimulator determined by a dye distribution in cadavers as well as anesthesia in living animals. We hypothesized that PBPB in sheep could be performed routinely resulting in sufficient dye distribution in nerve roots and a complete block of the entire thoracic limb with minimal adverse effects.

Materials and Methods

A total number of 13 Lori-Bakhtiari ewes with the age of approximately five months and apparent health were used. The sheep belonged to a private herd, keeping and growing up Lori-Bakhtiari sheep. The animals were transferred to the Veterinary Hospital at least 2 weeks prior to the commencement of the study and were held in the same husbandry and management conditions in indoor pens. Feed (balanced alfalfa, barley and straw supplemented with minerals) and water were provided twice a day and ad libitum, respectively. The health status of the sheep was confirmed via a thorough physical examination and complete blood count, total protein and fecal assessments. All the experiments were performed in the morning. No fasting and water limitation were applied before each experiment. The Animal Ethical Committee of Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz approved the investigation (EE/97.24.3.49899/scu.ac.ir).

Before the initiation of the study, the accessibility and identifiability of landmarks (sixth cervical vertebra (C6) and first thoracic vertebra (T1)) were evaluated and the technique was practiced in sheep skeleton and cadavers. The skeleton and cadavers had the same size as about a 20.00-kg sheep. The skeleton was used to observe and identify the special feature and probable location of C6. Then, it was recognized in cadavers via palpation of its sledge-like shape and smaller transverse process than the cranial and caudal transverse processes of the other vertebrae in the cervical region. A hypodermic needle (21-gauge, 3.80 cm; Supa Medical Device, Tehran, Iran) was inserted into the skin dorsomedially just above the transverse process until encountering the bony structure of the dorsal part of the transverse process of C6. Then, the needle was withdrawn (about 1.00 - 1.50 cm) and advanced obliquely (approximately 30.00 – 45.00 degree to the transverse process) first cranially and then caudally with the aim to access to C6 and C7 nerves, respectively. For T1 vertebra, the scapula was pulled caudally, the first rib was palpated and continued proximally to palpate the vertebra. The needle was directed in the same manner for C6 vertebra to the cranial and caudal border of the transverse process to target C8 and T1 nerves, respectively. An experienced anesthetist under the supervision of an anatomist performed all the procedures.

The main part of the present study was performed in two phases to determine dye distribution in cadavers (phase I) and onset and duration of anesthesia in living sheep (phase II). The complications were assessed in both the phases:

**Phase I.** Five ewes were selected randomly by drawing five out of 13 papers corresponding to each sheep from a closed box. The animals received a solution containing 1:1 methylene blue (1.00% methylene blue; Merk, Darmstadt, Germany) and lidocaine (2.00% lidocaine hydrochloride; Caspian Tamin, Tehran, Iran) at the target sites for PBPB. Sheep were positioned in right lateral recumbency (the left side was uppermost) and restrained manually. An area from midcervical to the caudal border of the scapula was clipped and aseptically prepared (7.50% povidone iodine; Daru Darman Salafchegan, Tehran, Iran). The transverse process of C6 vertebra and the first rib and then, the transverse processes of T1 vertebra were identified by palpating the landmarks using the same method employed for cadavers. The structures were marked with a marker pen. An electrical nerve stimulator (Stimulex HNS11; Pajunk, Geisingen, Germany) was used to guide the needle. The negative electrode of the nerve stimulator was connected to an insulated needle (22-gauge, 5.00 cm; Pajunk) and the positive electrode was attached to the skin via a transcutaneous electrical nerve stimulation (TENS) pad at the lateral side of the chest wall. The syringe containing the solution was connected to the insulated needle via an extension line which was prefilled with a 0.40 mL of the solution. The insulated needle was then inserted dorsomedially to the skin and directed to the transverse process of C6 vertebra until encountering the process. Then, the needle was withdrawn (about 1.00 - 1.50 cm) slightly and directed obliquely to the cranial and caudal borders of the transverse process. At the borders, the nerve stimulator was turned on and advanced forward for targeting C6 and C7 nerves, respectively. The nerve stimulator was initially set at 1.00 mA, 0.20 msec and 2.00 Hz. By observing the muscle twitches, the needle was moved in the place to exert the highest twitches with the lowest current (not below 0.20 mA). After negative aspiration of blood or air, a 1.50 mL of the solution was injected slowly. After accomplishing the procedures for C6 and C7 nerves, the needle was withdrawn and moved forward to the cranial and caudal border of T1 vertebra. Then, the C8 and T1 nerves were located and the injection procedures were repeated as previously described. Inward or outward rotation of the shoulder for C6 nerve, inward or outward rotation of the brachium for C7 and extension of the elbow for C8 and flexion of the carpus and digits for T1 were considered as the desired muscle twitches when the nerve stimulator was in the close contact to the nerve roots. For the nerves that required higher currents (more than 0.50 mA) to exert desired muscle twitches, the needle was very slightly moved in a fan-like shape at the time of injection. If hiccup and/or respiratory disturbances were observed during needle
advancement, it was eliminated by needle redirection and/or reorientation. The time of injection, which was defined from needle insertion to the skin for blocking C6 nerve to removing the needle from the skin after injection for T1 nerve, was recorded. Following the injections, the animal was positioned in sternal recumbency for 15 min and then was allowed to stand. The sheep were humanly slaughtered immediately and an area from the neck to the middle portion of the abdomen was separated. The anatomical area of C5 to T1 was dissected and the nerve roots were located. The nerves comprising the brachial plexus were also identified. The lengths of circumferential dye staining in the nerve roots were measured using a calibrated caliper (Guanglu Measuring Instrument Co., Ltd., Guilin, China). The brachial plexus nerves at the scapular region were also dissected and evaluated for dye distribution. The phrenic nerve pathway from cervical area to the thoracic region was also followed and assessed.

A laminectomy was performed at cervical and thoracic vertebral canal and the possible dye spread to the epidural space was determined.

**Phase II.** Eight remaining living ewes with the technique carried out as in the previous phase for blocking C6, C7, C8 and T1 nerves were used. A solution containing 2.00% lidocaine was injected at each site until the muscle contractions disappeared. The time to the onset of sensory block was assessed at four skin sites corresponding to axillary (AX), musculocutaneous (MC), radial (RA) and ulnar (UL) nerves (Fig. 1) using pin-prick and pinching tests. Pin-prick tests were applied with a 25-gauge needle pricking at the depth of approximately 1.00 mm and 3.00 – 5.00 mm for superficial and deep evaluations, respectively. A Rochester hemostat (20.00-cm Rochester Dean hemostatic forceps; Martin Group, Tuttlingen, Germany) closed at the first ratchet for 2 sec was used for pinching assessments. The sensitivity of both the thoracic limbs to skin tests was evaluated before injection of the solutions. The onset of sensory block was identified when responses to the both tests applied every 30 sec after the completion of the injections were negative. The time to the onset of motor block was defined when the animal was not able to bear its own weight at the injected limb. The sensory and motor blocks were evaluated every 10 min using aforementioned tests to determine the duration of anesthesia. The response to the sensory stimulations were scored as 0- no block (vigorous response including limb withdrawing and kicking), 1- mild block (less vigorous response including slow limb withdrawing and avoidant movement), 2- moderate block (substantially diminished response including muscle twitches and turning back the head toward the stimulated site) and 3- complete block (no response to stimulation). Only if no response to superficial pinpricks was observed, a deep muscular pinprick and hemostat pressure were applied. To diminish the possibility of skin damage, the needle or hemostat tests were done at slightly different sites for each time point. For determining the duration of motor block, the animal was encouraged to bear its own weight on standing position. Heart rate (HR), respiratory rate (RR) and rectal temperature (RT) were recorded at base (before performing the block when the animal was in standing position) and at 5, 10, 15, 20, 30, 45, 60, 75, 90, 105 and 120 min after drug administration. The HR was measured via chest auscultation at the left side of chest wall (fourth intercostal space, behind the olecranon). The RR and RT were recorded through thoracic and abdominal excursion and using a digital thermometer (AEG, Berlin, Germany) contacted to the rectal mucosa, respectively. Ketoprofen (1.10 mg kg⁻¹; Razak, Tehran, Iran) was administered after the experiment intramuscularly once a day for three days. All the injections were done by one investigator and all dissections were performed by another investigator. The length of dye staining in the nerves were measured by two independent investigator and the arithmetic average was reported. All the evaluations of HR, RR and RT were performed and recorded by one investigator.

**Statistical analysis.** The data were analyzed using SPSS Software (version 25.0, IBM Corp., Armonk, USA). Normal distribution of data was checked by a Shapiro-Wilk test. The length of staining among C6-T1 in phase I, the volume of administered lidocaine and the onset and duration of anesthesia among the evaluated cutaneous zone in phase II were compared using One-Way ANOVA and Bonferroni’s post hoc when needed. The scores among cutaneous zone were compared using Kruskal-Wallis test. A repeated-measure for ANOVA was employed for the comparison of HR, RR and RT over time. Parametric and nonparametric data were presented as mean and SD and median (minimum-maximum), respectively. Statistical significance was considered when \( p < 0.05. \)
Results

Phase I. The mean ± SD body weight of five used sheep was 20.10 ± 2.20 kg. All sheep received 6.00 mL solution of lidocaine-methylene blue, which is equal to 0.30 ± 0.03 mL kg⁻¹. Nerve injections was accomplished within 5 min. The currents applied for muscle twitching were 0.4 mA for C6 and C7, 0.40 - 0.50 mA for C8 and 0.50 - 0.70 mA for T1. In the first sheep, inability to bear weight on both the thoracic limbs was observed. Anatomical assessments in this sheep revealed dye spread into the vertebral canal. In the other four sheep, bilateral motor block and/or staining of vertebral canal were not detected. Anatomical evaluation of the brachial plexus showed that, in all sheep, brachial plexus is comprised of ventral branches of C6-T1 nerves and C5 and T2 did not contribute to brachial plexus formation. The C6-T1 nerves were stained in four out of five sheep (Fig. 2A). Ventral branches of C6-T1 were not stained in the first sheep that the dye spread into the vertebral canal. The lengths of staining were significantly higher for C6 (61.75 ± 5.50 mm) and C7 (72.75 ± 9.18 mm) nerves in comparison with C8 (40.75 ± 2.99 mm) and T1 (17.75 ± 3.30 mm) nerves (p ≤ 0.002). The length of dye staining in T1 was lower than the other three nerves (p ≤ 0.001). In all sheep, the origin of nerves at the intervertebral foramen were stained except for T1 in which dye was observed 7.00 - 10.00 mm distal to the origin. Dye distribution was not observed in the brachial plexus nerves in the axillary region. The long thoracic nerve at the lateral side of serratus ventralis muscle was also intact. The phrenic nerve originated from C5 and C6 was also intact in both cervical and thoracic regions (Fig. 2B). Dye diffusion into the cranial mediastinum was observed in three out five sheep (Fig. 2C). Diaphragmatic contraction characterized by hiccup and respiratory disturbances was observed in two out of five sheep, which discontinued when the needle was redirected and/or reoriented.

Phase II. The mean ± SD body weight of the eight sheep used in this stage was 19.80 ± 2.80 kg. The total volume of administered lidocaine was 4.40 ± 1.20 mL for each sheep which was equal to 0.24 ± 0.10 mL kg⁻¹ and 4.90 ± 2.00 mg kg⁻¹. The volumes of administered lidocaine were 0.90 ± 0.30 mL for C6, 0.90 ± 0.20 mL for C7, 1.00 ± 0.20 mL for C8 and 1.20 ± 0.30 mL for T1 (p = 0.19). The duration of accomplishing the injections was about 7 min with the longest duration allocated to locating T1 nerve. The required currents for eliciting muscle twitches were 0.40 mA for C6 and C7, 0.40 - 0.50 mA for C8 and 0.50 - 0.70 mA for T1. In two out of eight sheep, the sensory and motor block were not observed and the block was repeated the following week. In three out of eight sheep, despite complete block at AX and MC, sensory block was not observed at RA and UL. The time to the onset of sensory block was less than 10 min for all skin tests. All sheep with successful block of AX and MC (regardless to the RA and UL block) were not able to bear the weight at the time of standing within 15 min following the injections. No significant differences were found among the duration of sensory blocks among cutaneous zone as well as motor block (p = 0.28; Fig. 3). Mean durations of sensory (including complete and partial) and motor blocks were 60.70 and 76.40 min, respectively. Comparison of the given scores showed higher values for AX [2 (2-3)] and MC [2 (1-3)] than those of UL [1 (1-2)] and RA [1(1-2)] at 40 min after the onset of block (p = 0.04; Fig. 4). The success rate of AX and MC blocks were 75.00%, and it was 37.50% for RA and UL. With respect to HR, RR and RT, no significant changes were observed between baselines (87.00 ± 9.00 beats per min, 35.00 ± 5.00 breaths per min and 39.80 ± 0.50 °C) and time point values (mean: 87.00 ± 14.00 beats per min, 33.00 ± 3.00 breaths per min and 39.40 ± 0.60 °C; p > 0.05). In one sheep, persistent flexion of the fetlock joint and inability of weight bearing were observed after the injection approximately up to 4 hr later.

Fig. 2. A) Dye distribution at the emergence of spinal nerves (C6-T1), B) Lack of dye along the thoracic part of phrenic nerve (white arrows), and C) Dye spread into the cranial mediastinum (black arrow) following paravertebral brachial plexus block in sheep receiving a solution containing 1:1 methylene blue 1.00% and lidocaine 2.00%, * = 3.00 cm scale bar.
The success rate of AX and MC or locating nerves belonged to T1 and C6 was acceptable for C8 involved at acceptable landmarks palpation, successful block (defined as dye staining at the intervertebral foramen) of all four nerves were observed in 33.00% of cases. The authors stated that addition of a nerve stimulator might increase the success rate.

Addition of a nerve stimulator might increase the success rate of brachial plexus formation (i.e., C6 nerve) in dogs, Campoy et al. considered five times more of the mentioned length (i.e., 2.00 cm) as sufficient for a nerve block. The researchers of the previous study also arbitrarily chose staining of more than 4.00 cm as over-staining. Considering the study by Campoy et al., in the current investigation, the staining rates in C6 and C7 nerves were higher, in C8 nerve was acceptable and in T1 nerve was less than that required for complete nerve block. In other words, the used volume (i.e., 1.50 ml per site) was high for C6 and C7 nerves, acceptable for C8 nerve and low for T1 nerve.

Cutaneous zones of AX, MC, RA and UL were used for evaluation of C6 to T1 nerve anesthesia. Given that AX nerve originates from C6 and C7, MC nerve from C6, C7 and C8, RA nerve from C7, C8, and T1, and the UL nerve from C8 and T1, the skin tests can evaluate C6 to T1 nerves. Block success rate in AX and MC was 75.00% and in RA and UL it was 37.5%. Consistently, Choquette et al. reported a block success rate of 89.00% to 11.00% with a decreasing trend from the upper to the lower parts of the limb in dogs. The researchers attributed the results to the specific anatomical location of the nerves, with the ultrasound observation of 90.00% for C6 and only 32.00% for C8-T1. Similarly, in the present study, finding T1 nerve was the most challenging part of the paravertebral block.

**Discussion**

In the present study, in phase I, performing BPB in the left thoracic limb via paravertebral approach resulted in the dissimilar staining of all the nerve roots (i.e., C6-T1) contributed to brachial plexus formation in four out of five sheep. Dye staining into the cranial mediastinum was observed in three out of five sheep. In phase II, sensory and motor blocks started within 10 and 15 min, respectively. Mean durations of sensory (including complete and partial) and motor blocks were 60.70 and 76.40 min, respectively. The success rate of AX and MC blocks were 75.00%, and it was 37.50% for RA and UL.

In a study in dogs submitted to receive BPB with just landmark palpation, successful block (defined as dye staining at the intervertebral foramen) of all four nerves were observed in 33.00% of cases. The authors stated that addition of a nerve stimulator might increase the success rate.

In the current study, all the nerves contributing to brachial plexus formation (i.e., C6-T1) were stained in four out of five sheep following BPB (i.e., success rate of 80.00%). The BPB failed and the nerves were not stained in one sheep most probably because of wrong technique.

![Fig. 3. Duration of sensory block (mean ± SD) at the sites of skin tests for axillary (AX), musculocutaneous (MC), radial (RA) and ulnar (UL) nerves, and motor block (paralysis) in 8 sheep following paravertebral brachial plexus block with 2.00% lidocaine.](image)

![Fig. 4. Changes in the scores (0: No block; 3: Complete block) at the sites of the skin tests for axillary (AX), musculocutaneous (MC), radial (RA), and ulnar (UL) nerves in 8 sheep following paravertebral brachial plexus block with 2.00% lidocaine. *Significantly different from RA and UL (p < 0.05).](image)
and in most cases, it required higher currents than those for C6, C7, and C8 nerves to elicit muscle contraction response. As stated in the phase I, due to the specific anatomic position of T1 nerve, the needle might not be able to target the nerve well, and the muscular response was detected only using high currents.

The mean duration of sensory block in the present study was ranged from 55 to 71 min. Although there were no significant differences between the durations of sensation loss for different nerves, the highest duration was for AX nerve and the lowest for RA and UL. In the present investigation, the average durations of sensory and motor blocks were 62.8 and 76 min, respectively. In the study of the brachial plexus block with lidocaine using the paravertebral approach in dogs, Chequette et al. reported an average time of 82 and 118 ± 63 min for sensory and motor blocks, respectively. The higher mean anesthesia time in the mentioned study can be attributed to higher doses of lidocaine (6.00 mg kg⁻¹) than those in the present study (4.90 mg kg⁻¹). Moreover, the length of motor block in the study by Chequette et al. was determined based on step disorder rather than weight bearing. The discrepancies in species could also be considered as determining factors.

A number of complications associated with paravertebral brachial plexus block were described such as spinal, or epidural anesthesia, brain stem poisoning, Horner syndrome, hiccup, and unilateral diaphragmatic paralysis due to phrenic nerve involvement. Rioja et al. reported methylene blue dye accumulation around the spinal cord in 29.00% - 39.00% of cases following PBPB in dogs. Epidural space staining has also been reported after PBPB in dogs. In this study, dye spread to vertebral column was observed in the first sheep of phase I. Given that the nerve roots were not stained in this case, presumably the presence of medication in the vertebral column was due to a mistake in the injection technique which was corrected in the next injections.

The most prominent complication of PBPB in the current study was needle perforation and drug injection into the anterior thoracic space, probably as a result of attempting to block T1 nerve. It should be noted that in the current investigation, if it required higher currents to exert desired muscle twitches, the needle was moved in a fan-like shape at the time of injection to ensure adequacy of the block. It is possible that this maneuver was resulted in this complication. Perforation of the thoracic space and discoloration of the visceral pleura and intercostal space following PBPB have also been reported in dogs. It has been suggested that the use of a modified paravertebral approach will prevent this complication. In addition, in the phase II of the present study, hiccups and difficulty to breathe were detected in some cases resolved by relocating the needle.

In the present study, PBPB was able to block the upper and the lower parts of the elbow joint (completely or partially) in the forelimb. In the previous studies in sheep, just the areas below the elbow were blocked via the axillary approach (i.e., ABPB). The paravertebral approach, the final volume of the injected lidocaine was about 0.24 mL kg⁻¹, which was comparable to 0.25 mL kg⁻¹ and was less than 0.30 mL kg⁻¹ compared to the volumes used for ABPB in sheep. The mean durations of sensory and motor blocks following PBPB were 61 and 76 min, respectively, whereas, durations of approximately 100 and 89 min for sensory and 111 min for motor blocks have been reported following ABPB in sheep. It is obvious that for a more specific comparison between APBP and PBPB in sheep, the established methods of both techniques may be compared in future investigations.

Some limitations of the current investigation should be addressed. First, although prior to the main study the correct and effective performance of PBPB was practiced, the results might have been due to the lack of insufficient expertise in doing PBPB in sheep. Second, due to the stoic nature of sheep, employing the response to noxious stimuli as an indicator for pain perception might not be adequate and be associated with some false negative outcomes in these species. It is of importance especially when in the current investigation noxious stimuli were applied just for the left limb and was not compared to the contralateral limb as the control. Third, the results might not be translatable to the other breeds of sheep, sheep with different ages because of variation in body conformation, size, fat content and variation in the nerves anatomy.

Brachial plexus block with the paravertebral approach in the left thoracic limb of sheep, although provided an acceptable block for the upper part of the elbow joint, resulted in reduced success rate and lower block duration for more distal parts of the limb. The paravertebral BPB in the left thoracic limb of sheep seems not effective for the inferior part of the elbow joint and may be associated with some complications for the animal. Further studies are required to determine whether combination of various approaches and/or employing the modified technique would improve the success rate and diminish the complications of BPBP in sheep.

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Conflict of interest

The authors declare no conflict of interest.
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