COMPARATIVE EVALUATION OF ULTASOUND GUIDED PERINEURAL AND COLOR-DOPPLER GUIDED PERIVASCULAR BRACHIAL PLEXUS BLOCKS IN SHEEP

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ABSTRACT

Twenty-four adult sheep irrespective of sex, randomly allotted to four groups i.e., A, B, C & D with six animals each formed the subjects of study with the objectives to identify the window most feasible for localization of brachial plexus in adult sheep and its subsequent blockade under ultrasonographic guidance and color doppler; to compare the perineural brachial plexus blockade (BPB) using ultrasound guidance and perivascular BPB using color doppler and to ascertain possible dose regimen variation and anesthetic effect with and without isoflupredone acetate. All the animals received 0.75% ropivacaine hydrochloride @ 3mg/kg BW. Perineural ultrasound guided BPB was performed in animals of group A & B, in which group A animals received ropivacaine whereas group B animals received additional 4mg isoflupredone, perivascular colour doppler guided BPB was performed in groups C & D, in which received ropivacaine and ropivacaine plus 4mg isoflupredone respectively. We identified the appropriate scanning window. The perivascular BPB allowed a feasible and accurate access to BP and significantly shortened the onset and prolonged the duration of anesthesia as compared to perineural BPB. By incorporating 4mg isoflupredone, ropivacaine dose was reduced by 15mg per animal and it produced early onset and prolonged duration of anesthesia.
I. INTRODUCTION

In ruminants, surgical procedures are commonly performed under sedation and local or regional anesthesia and are preferred over general anesthesia in these animals because they produce minimal cardiopulmonary alterations, require limited amount of equipment, minimize veterinary supervision, lower the cost of the procedure [21], facilitate clinical work with the animal in the standing position, while providing analgesia with minimal adverse effects [13] and avoid other known complications of general anesthesia. However, disadvantages may include the difficulty in identifying anatomic landmarks, variability in anatomic pathways of peripheral nerves, risk of penetrating into other structures or the large volume of local anesthetic. The variable anatomy between individuals may lead to poor success rates for specific peripheral nerve blocks [22,17].

To overcome such difficulties, ultrasound and peripheral nerve stimulation guided techniques of local anesthesia have been started in animals. For the past two decades, the electrical nerve stimulator has been the gold standard for nerve localization in regional anesthesia [9,12,4,1] but the nerve stimulation may not always elicit a motor response and doesn’t guarantee success [18]. However, with recent developments in high-frequency imaging, the use of ultrasound technology has significantly increased for nerve localization [20,14,26,2]. The ultrasound guided technique offers reported additional advantages, including avoidance of intraneuronal/intravascular injection, faster onset times, improved block quality, decreased pain from muscular contractions, prolonged postoperative analgesia, and decreased need for rescue analgesics [16,25,23,27].

Local anesthetics alone provide analgesia for not more than 4-8 hrs., so various additives like opioids[24], clonidine [5], hylase etc. were added to local anesthetics, but the results are either inconclusive or associated with side effects [15]. Steroids have powerful anti-inflammatory as well as analgesic property. Perineural injection of steroids is reported to influence post operative analgesia; hence injection isoflupredone acetate along with ropivacaine, a long acting local anesthetic agent was used in the present study. Steroids relieve pain by reducing inflammation and blocking transmission of nociceptive C-fibres and by suppressing ectopic neural discharge [8].

Brachial Plexus Block was first described by Tufvesson (1951) as a means of providing anesthesia of the forelimb in dogs [6]. Abramowitz and Cohen (1981) described the first use of doppler ultrasound to identify the axillary artery, thereby aiding the performance of axillary plexus block for upper limb surgery [7]. But it was the use of B-mode ultrasound (Ting and Sivagnanaratnam, 1989) for axillary block performance that heralded the era of ultrasound-guided peripheral nerve block [19]. Sheppard et al. (1998) evaluated the ability of ultrasound to visualize components of the brachial plexus, using MRI as a guide to background anatomy. They described the plexus as having a hypoechoic appearance within hyperechoic rims which were tubular on longitudinal scans and oval to round on transverse scan they also felt that color doppler was essential to prevent the confusion of nerves with small blood vessels [3]. Yang et al. (1998) studied anatomy of the brachial plexus under ultrasound and subsequently used it to guide the placement of catheters for interscalene and supraclavicular blocks for an arm surgery in humans [28].
Campoy et al. (2010) described an ultrasound guided technique for axillary brachial plexus, femoral and sciatic nerves in dog. Location of the transducer in the axilla produced images of the axillary blood vessels. Axillary artery was identified by its characteristic anechoic pulsatile ultrasound image. Three rounded hyperechoic structures were observed dorsal and close to the axillary vessels presumed to be the C7, C8, and T1 roots of the brachial plexus [11]. According to Carter et al. (2011), ultrasound guided interscalene block target the roots and proximal trunks of the brachial plexus as they are sandwiched between the anterior and the middle scalene muscle [10].

Keeping the scenario in view the present study was planned with the following objectives:

- To identify the window most feasible for localization of brachial plexus in adult sheep and its subsequent blockade under ultrasonographic guidance and color doppler.
- To compare the perineural brachial plexus blockade using ultrasound guidance and perivascular brachial plexus blockade using color doppler.
- To ascertain the possible dose regimen variation and anesthetic effect with and without isoflupredone acetate.

II. MATERIALS AND METHODS

The study was conducted at Mountain Research Station for Sheep and Goat (MRSG) / Division of VSR, F.V.Sc & AH, SKUAST - Kashmir.

1. Animals of the study
The study was conducted on 24 adult sheep irrespective of sex in the age group of six months to one year. The animals were housed under similar managemental conditions at the research centre since birth. The animals were divided randomly in four groups viz. Group A, B, C and D comprising six animals each.

2. Instrumentation
An ultrasound system TELEMED CAB with a 5-10MHz linear transducer was used for ultrasound guided brachial plexus perineural and perivascular block in sheep in lateral recumbency (Fig. 1).

III. PREPARATION OF THE PATIENT
Before administering any drug, the sheep were subjected to overnight fasting for 12 hours. All animals underwent antisepsis of the appendages prior to brachial plexus block (Fig. 2).
IV. TECHNIQUE USED FOR BRACHIAL PLEXUS BLOCK

4.1 Brachial plexus block in Group A animals
The animals were restrained in lateral recumbency which was followed by application of copious gel over the prepared site. After standardization of procedure from different possible angles and borders of scapula and anatomical area in vicinity the window was identified. The exact area where from the brachial plexus was visible was by placing the transducer along the medial aspect of scapula over the triceps and latissimus dorsi muscle (Fig. 3). The axillary lymph node was identified and the needle was inserted under the guidance of ultrasound scanner. The needle was slowly pushed forward above the level of axillary lymph node so that the bevel of the needle was nearer to the plexus close to the radial nerve and the anesthetic agent i.e. 0.75% ropivacaine hydrochloride was injected and its spread around the brachial plexus was clearly monitored on the screen of the ultra sound scanner. Ultrasonography was performed by using TELEMED CAB with a 5-10MHz linear transducer. 0.75% ropivacaine hydrochloride@ 3mg/kg b.wt. was deposited near the brachial plexus slowly in instalments (Fig. 4). The deposition of the anesthetic agent at brachial plexus was monitored on USG screen.

4.2 Brachial plexus block in Group B animals
In Group B, the procedure performed was same as that in group A. Ultrasonography was performed by using TELEMED CAB with a 5-10MHz linear transducer. The animals were subjected to brachial plexus blockade using a combination of 0.75% ropivacaine hydrochloride@ 3mg/kg b.wt. and 2ml (4mg) isoflupredone acetate. However addition of 2 ml (4 mg) isoflupredone acetate replaced 2 ml (15 mg) of ropivacaine hydrochloride, thereby reducing the dose of latter by 15 mg.

4.3 Brachial plexus block in Group C animals
In this group of animals, brachial plexus was blocked by using perivascular ultrasound guided block technique. Ultrasonography was performed by using TELEMED CAB by placing transducer on the triceps brachial muscle. The axillary area was then scanned with the transducer orientated in a parasagittal plane, the transducer was glided, rotated or tilted until an optimal short axis (transverse) view of the axillary vessels was obtained. The axillary artery was identified by its characteristic anechoic pulsatile ultrasound image (Fig. 5). After confirming that blood could not be aspirated and that there was minimal resistance to injection, calculated dose of 0.75% ropivacaine hydrochloride @ 3mg/kg BW was deposited around the artery (Fig. 6).
In Group D, the procedure performed for ultrasound scanning was same as in group C. Calculated dose of 0.75% ropivacaine hydrochloride @ 3mg/kg b.wt. and 2ml (4mg) isoflupredone acetate was deposited around the axillary artery. However addition of 2 ml (4 mg) isoflupredone acetate replaced 2 ml (15 mg) of ropivacaine hydrochloride, thereby reducing the dose of latter by 15 mg. After the deposition of anesthetic agent the needle was withdrawn slowly, and isopropyl alcohol swab was kept in position for two minutes. The effect of the anesthesia was monitored for 360 minutes and parameters were recorded as under:

V. ASSESSMENT OF SENSORY BLOCKADE

Sensory blockade of the musculocutaneous, median, radial and ulnar nerves was assessed in the corresponding dermatomal areas. After the completion of the block procedure, sensory onset was considered when there was dull sensation to pin prick along the distribution of any of the above-mentioned nerves. The duration of sensory block was defined as the time interval between the end of anesthetic administration and the complete resolution of anesthesia on all nerves. Sensory blockade of musculocutaneous, median, radial and ulnar nerves was graded according to three-point numerical rating scale (NRS) using pin prick test:

Grade-0: Sharp pin sensation felt; Grade-1: Analgesia (dull sensation felt); Grade-2: Anesthesia (no sensation felt)

VI. ASSESSMENT OF MOTOR BLOCKADE

Motor blockade was assessed based on the degree of abnormal gait while walking and by observing abnormal clinical signs (Fig.s 7, 8, 9 and 10). The scoring was done on a three-point numerical rating scale (NRS) as follows:

Grade-0: Normal motor function/normal gait while walking and no abnormal sign while standing; Grade-1: Animal can walk while bearing mild to moderate weight and no abnormal sign while standing; Grade-2: Complete motor blockade with inability to bear weight.

Onset of motor blockade was considered when there was Grade 1 motor blockade after completion of block procedure. Peak motor block was considered at Grade 2 motor blockade. The duration of motor block was defined as the time interval between the end of local anesthetic administration and the recovery of complete
motor function of the forelimb. The block was considered as failed when analgesia to pin prick was not elicited at the site of surgical incision even after 30 minutes of drug administration. In all the groups, the block onset, duration and recovery were monitored by application of neuromuscular stimulator after calibration of the requisite frequency.

The onset and duration of sensory block, motor block and complications were noted at 5, 10, 15, 30, 45, 60, 75, 90, 105, 120, 150, 165, 180, 210 and 240 minutes and at 360 minutes post induction of nerve blocks.

VII. STATISTICAL ANALYSIS
The data was statistically analyzed by Duncan’s Multiple Range Test using software SPSS 20 and results were presented as mean ± SD. Statistical significance was defined as $P < 0.05$ and inferences were drawn.
VIII. RESULTS AND DISCUSSION

In this study 0.75% ropivacaine hydrochloride was used at the dose rate of 3mg per kg body weight based on the pilot trials conducted prior to study.

1. Group A (n=6): Brachial plexus blockade under ultrasound guidance

The site selected was near the caudal border and on the distal aspect of scapular area with the transducer placed on the long head of triceps muscle. The axillary lymph node was identified as landmark under USG scanning (Fig. 11, Fig. 12). A 5 inch 16 gauge needle was directed between thoracic wall and scapula through triceps above the level of axillary lymph node.

The first nerve to get anesthetized and desensitized was radial nerve at 5 min (0.50±0.55) post injection (Fig. 13). Blockade of radial nerve followed the anesthetic effect development in ulnar nerve which was desensitized at 10 minutes post injection. The effect significantly (p<0.05) increased at 15 minutes (1.50±0.55) and reached maximum at 30 minutes post injection (1.83±0.41). The effect non-significantly (p>0.05) decreased at 90 minutes (1.50±0.84), 120 minutes (1.33±1.03), 150 minutes (1.17±0.98) and 180 minutes (0.67±0.82) and lasted up to 210 minutes (0.50±0.55) post injection and thereafter no effect in the region could be noted. Pinpricking revealed desensitization of the median nerve was similar to that of ulnar nerve, it started at 10 minutes (0.17±0.41) and the effect increased significantly (p<0.05) at 15 minutes (1.17±0.75). The effect of anesthesia remained steadily non-significant from 30 minutes to 75 minutes post injection (1.83±0.41); thereafter the anesthetic effect decreased non-significantly (p>0.05) at 90 minutes (1.50±0.84), 105 minutes (1.33±1.03), 150 minutes (1.17±0.98) and the effected lasted up to 210 minutes (0.50±0.55) post injection beyond which no effect was seen in the animals of this group. The desensitization of the medial aspect in the distal third of humerus marked the desensitization of musculocutaneous nerve which started at 10 minutes (1.00±0.63). It non-significantly (p>0.05) increased at 15 minutes post injection interval (1.67±0.52) and got abolished after 240 minutes post injection. The signs of motor block namely, walking while bearing mild weight, abnormal posture, flexion of fetlock joint appeared at the 5th minute post injection (0.67±0.52) in the animals of group A. The effect significantly (p<0.05) was much pronounced clinically at 10 minutes (1.50±0.55) and the effect remained higher from 15 to 120 minutes (1.83±0.41), though non-significant (p>0.05). Thereafter, it non-significantly (p>0.05) decreased to 1.67±0.82, 1.50±0.84 and 1.17±0.98 at 135, 165 and 210 minutes post injection respectively. The duration of motor block and anesthesia for radial nerve was 235 minutes followed by anesthesia of musculocutaneous nerve (230 minutes) and ulnar and median nerves (200 minutes). The anesthesia
was completely abolished at 360 minutes. The application of transcutaneous electrical nerve stimulator at the calibrated frequency for 0.9 seconds revealed no response during the phase of anesthesia. However, animals of this group responded to the stimulation by transcutaneous electrical nerve stimulation at the preset frequency for 0.9 seconds which confirmed the return of reflexes after 360 minutes post injection.

2. Group B (n=6): Brachial plexus blockade under ultrasound guidance (Combination of 0.75% ropivacaine hydrochloride and injection isoflupredone acetate)

In the animals of group B, radial and musculocutaneous nerves were the first to get desensitized, however the radial nerve desensitization was more pronounced (Fig. 14). The onset of effect of the radial nerve was noted at 5 minutes post injection (0.83±0.75). The depth of desensitization significantly (p<0.05) increased at 15 minutes post injection (1.83±0.41). Up to 210 minutes post injection remained almost unchanged which followed abrupt and significant (p<0.05) decline (0.83±0.75) at 240 minutes post injection. The anesthetic effect further declined significantly (p<0.05) and mild effect was seen at 360 minutes post injection (0.33±0.52). The anesthetic effect of the ulnar nerve was evident at 10 minutes post injection (0.33±0.52) which increased significantly (p<0.05) at 15 minutes (1.00±0.63). This followed a non-significantly (p>0.05) declined trend from 180 minutes (1.50±0.55) to 210 minutes (1.00±0.63) and significantly (p<0.05) declined trend at 240 minutes (0.33±0.52) respectively. The anesthetic effect ended thereafter and at 360 minute post injection the animals were free from the effect of anesthesia as far the ulnar nerve is concerned. Median nerve desensitization in the animal of group B followed a similar pattern, desensitized at 10 and 15 minutes post injection with mean values of 0.17±0.41 and 0.50±0.55 respectively. The anesthetic effect increased non-significantly (p>0.05) at 20 minutes (1.17±0.75) and 30 minutes (1.50±0.84) post injection period followed by a non-significant (p>0.05) and constant increase at 45 minutes post injection (1.67±0.82) which continued up to 180 minutes. The anesthetic effect significantly (p<0.05) dropped to 0.50±0.55 at 210 minutes and the anesthesia lasted thereafter so that towards the terminal period of observation. The musculocutaneous nerve showed desensitization at 5 minutes (0.17±0.41) and the effect increased significantly (p<0.05) to 0.83±0.75 at 10 minutes and non-significantly (p>0.05) to 1.17±0.75 at 15 minutes respectively. The effect of anesthesia was however maximum from 20 to 165 minutes (1.83±0.41) and thereafter followed a non-significant (p>0.05) decline from 180 to 240 minutes post injection and no effect of anesthesia could be detected at 360 minutes. As far the degree of desensitization at 30 to 90 minutes is concerned both the groups A and B followed the same trends and the degree of depth (1.83±0.41), however the effect was rather less at 240 minutes in the musculocutaneous nerve of group A (0.33±0.52) than in the animals of group B at the same hour (0.83±0.75). Motor block could not be perceived in the animals of group B at 5 minutes post injection. It appeared at 10 minutes (1.17±0.41), significantly (p<0.05) increased at 15 minutes post injection (1.83±0.41) and remained steady up to 240 minutes. The depth followed a significantly (p<0.05) declining trend and decreased to 0.33±0.52 at 360 minutes post injections, so much so that the motor block did not abolish by 360 minute of observation. On comparing the duration of sensory and motor blockade in group B animals, the duration of sensory blockade was more and radial nerve was desensitized for maximum duration (355 minutes) followed by musculocutaneous nerve (235 minutes), ulnar nerve (230 minutes) and finally the median nerve (200 minutes). However, the duration of motor effect was 350 minutes. As far the comparative
degree of the anesthetic effect in the motor block in group A and B goes, both the groups showed similar depth and the pattern from 15 to 120 minutes post injection.

3. Group C (n=6): Ultrasound guided perivascular brachial plexus blockade

Fig. 1 compares the variation in sensory and motor responses at different time intervals in animals of group C. The onset of sensory analgesia was observed at 5 minutes post injection. When compared with the radial nerve desensitization of groups A, B and D, the effect deepened with the passage of time and continued to remain constant from 15 to 90 minutes (1.83±0.41); the anesthetic effect started decreasing non-significantly (p>0.05) at 105 minutes (1.67±0.82) and at 150 minutes (1.50± 0.84). The radial nerve was free from the effect of anesthetic effect after 240 minutes. In this group of animals blockade of ulnar nerve followed a similar pattern with the onset with mild effect at 5 minutes (0.17±0.41); peak effect between 30 to 75 minutes (1.83±0.41). The effect decreased non-significantly (p>0.05) to 1.67±0.82 at 90 minutes, 1.50±0.84 at 135 minutes, 1.17±0.75 at 165 minutes, 1.00±0.63 at 180 minutes and the effect lasted at 240 minute (0.67±0.52). Sensory anesthesis in median nerve of group C animals showed onset at 10 minutes post injection (0.83±0.41). The effect non-significantly (p>0.05) varied at 15 and 20 minutes post injection with mean values of 1.33±0.82 and 1.67±0.82 respectively. The peak effect was observed at 30 minutes (1.83±0.41) which lasted till 90 minutes, after that the effect got decreased non-significantly (p>0.05) and lasted for about 210 minutes and no effect could be detected beyond that hour of observation. In animals of group C, the musculocutaneous nerve desensitization showed effect with mean value 0.50±0.55 at 5 minutes post injection. The depth remained highest only between 20 to 75 minutes (1.83±0.41) and the anesthesia lasted up to 240 minutes. When compared with other groups, the musculocutaneous nerve remained desensitized for a longer duration (235 minutes) compared the animals of Group A (230 minutes). The onset of motor blockade initiated in the animals of group C at 5 minutes which significantly (p<0.05) increased at 10 minutes and continued increasing non-significantly (p>0.05) till 165 minutes (1.83±0.41); the anesthetic effect slowly decreased non-significantly (p>0.05) and showed a mean value 1.50±0.84 and 1.00±0.63 at 180 and 210 minutes respectively. The effect lasted up to 240 minutes.

4. Group D (n=6): Ultrasound guided perivascular brachial plexus block (Combination of 0.75% ropivacaine hydrochloride and injection isoflupredone acetate)

Ultrasound guided perivascular blockade in the animals of Group D revealed the onset of sensory and motor blockade both at 5 minutes (Fig. 16). Onset of radial nerve anesthesia was at 5 minutes post injection with the mean value 0.50±0.55, the effect non-significantly (p>0.05) increased up to 20 minutes and continued to remain non-significantly (p>0.05) higher at 30 to 120 minutes (1.83±0.41). The effect was over at 240 minutes. The duration of anesthetic effect was similar to the animals of groups A and C. No anesthetic effect of radial nerve was noted beyond 240 minutes. Similar trend was followed by the anesthetic effect on ulnar and median nerve with the onset at 5 minutes and duration of anesthesia up to 235 minutes with the peak effect at 45 minutes (ulnar) and 20 minutes (median). The musculocutaneous nerve was desensitized at 5 minute with a mean value 0.33±0.52. The peak effect of the anesthesia was recorded between 45 and 75 minutes (1.83±0.41). After 210 minutes, the effect was non-significantly (p>0.05) decreased up to 360 minutes (0.33±0.52). In the animals of Group D, signs of motor blockade were marked with a mean value 0.67±0.52 at 5 minutes post injection. The effect increased significantly (p<0.05) at 10 minutes (1.67±0.52) and the peak motor block was recorded from
20-180 minutes (1.83±0.41). The motor block effect continued till 240 minutes post injection with a mean value of 1.67±0.82. Thereafter no motor blockade was detected and animals walked with normal weight bearing.

Fig. 13: Desensitization pattern of different nerves and variation in motor response at different time intervals in animals of group A.

Fig. 14: Desensitization pattern of different nerves and variation in motor response at different time intervals in animals of group B.

Fig. 15: Desensitization pattern of different nerves and variation in motor response at different time intervals in animals of group C.
The overall results of the present study led to the following conclusions:

- The identified ultra scanning window allowed a feasible and accurate access to brachial plexus in the sheep and significantly shortened the onset and prolonged the block duration thereby decreasing the dose of anesthesia when compared with traditional brachial plexus block.

- Perivascular brachial plexus blockade using color doppler significantly shortened the block onset time and prolonged the block duration when compared with the perineural brachial plexus blockade using ultrasound guidance.

- In case of long acting anesthesia by incorporating 2ml of isoflupredone, we have been in a position to reduce the dose of ropivacaine by 15mg per animal. However, it has produced early onset and prolonged duration of anesthesia. Therefore, it could be concluded that this anesthetic regimen when used in brachial plexus block in sheep can form a very good anesthesia for forelimb surgery of four hours duration.

It can be concluded that, color doppler enables more safe and successful nerve block in a short time and prolongs the block duration when compared with perineural brachial plexus blockade using ultrasound guidance. All animals demonstrated a complete recovery, and no sequelae were recorded. Moreover, absence of any apparent sign of cardiac or central nervous system toxicities may be attributed to the efficacy of brachial plexus block. Clinical studies are needed to definitively demonstrate its clinical benefits.

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