



Research Article

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Surgical Analgesia, Physiological Responses and Cortisol Level in Goats Subjected to Exploratory Laparotomy under Distal Paravertebral Nerve Block, Conventional Needle Acupuncture and Aquapuncture



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Abstract

The study was conducted to explore an alternative method of producing analgesia for surgical procedure in goats. Eighteen apparently healthy 1-3 years old female Philippine native goats, weighing 10-20 kg were divided randomly into three groups with six animals each and subjected to the following:

- Distal paravertebral nerve block (DPNB) using lidocaine hydrochloride;
- Conventional needle acupuncture (CNA) through needling of Acupoints BA04, 31, 22, 26 and 47; and
- Aquapuncture using 2% chili pepper decoction (AQP) to stimulate the same acupoints as in CNA.

Exploratory laparotomy was conducted and analgesia, physiological responses and cortisol level in the animals were determined. Good to excellent surgical analgesia was produced in all six sheep of DPNB, five animals in AQP and one animal in CNA. Heart rate, respiratory rate and temperature were within normal levels in all groups, although higher values were observed in CNA and AQP than in DPNB. A higher than normal cortisol value was recorded in all treatment groups perioperatively. The results suggest that aquapuncture using chili pepper decoction can be used as an alternative to distal paravertebral nerve block in producing surgical analgesia for laparotomy in goats.

Keywords: Acupuncture; Analgesia; Aquapuncture; Cortisol; Goat; Regional analgesia

Abbreviations: CNA: Conventional needle acupuncture; AQP: Aquapuncture using chili pepper decoction; DPNB: Distal paravertebral nerve block; DOST-ASTHRP: Department of Science and Technology's Accelerated Science and Technology Human Resource Program

Introduction

In ruminants, exploratory laparotomy can be used both as a diagnostic tool and a treatment tool in various diseases affecting the abdominal organs Pugh and Baird [1]. It ensures a more precise diagnosis, prognosis and treatment protocol because it allows thorough palpation and examination of the affected organs Radostits [2]. It also complements physical examination, clinical pathologic examination and diagnostic imaging in management of undiagnosed cases Ames [3]. In goats, regional analgesia is the commonly preferred method for administration of anesthesia during surgery. It requires minimal instrumentation, with ease of drug administration and lesser cost. Since the animal is conscious during the procedure, the normal

digestive processes of the ruminant are maintained, thereby lessening the complications which might occur during and after the surgery Fubini & Ducharme [4]. Regional anesthesia can be achieved by administering the analgesic agent through local infiltration, inverted L-block, par vertebral block or epidural analgesia. Lidocaine hydrochloride, which is the commonly used analgesic agent, is cheap and locally available but presents some drawbacks. It must be used with caution among patients with severe respiratory depression or bradycardia and most of the time; patients presented for surgery have some of these clinical signs. Goats are sensitive to the effect of lidocaine and some practitioners suggested that the concentration of the drug

be reduced by adding saline to produce a 1% concentration or even a 0.5% concentration [5]. The doses should not also exceed 6mg/kg and redosing of the patient during extended period of surgical operation could increase the risk of toxicity Smith and Sherman [6].

To lessen the possible side effects on the use of anesthetic drugs particularly on patients with preexisting conditions, the use of alternative methods such as acupuncture has been studied. Conventional needle acupuncture was found to produce comparable analgesia as that of xylazine-ketamine anesthesia in sheep Acorda [5]. Comparison of analgesic effects of conventional needle acupuncture, hypodermic needle acupuncture, pneumoacupuncture and aquapuncture in sheep revealed that aquapuncture has comparable analgesic effect as that of conventional needle acupuncture Acorda [5]. In buffaloes, analgesia produced by electroacupuncture is similar to that of analgesia produced by local infiltration of lidocaine. The use of chili pepper (*Capsicum frutescens*-) decoction in aquapuncture has proven to give the highest percentage of analgesia among other solutions used in the study conducted by Acorda [5] in sheep but its effect has not been proven through actual conduct of surgery [5]. This study aims to explore a cheaper method of inducing analgesia for surgical procedure using a locally available product. The aim of this study is to evaluate the response of goats during exploratory laparotomy based on surgical analgesia produced, physiological responses of the animals and cortisol level using distal paravertebral nerve block, conventional needle acupuncture and aquapuncture. If acupuncture can be proven effective in producing surgical analgesia, it can be considered by practitioners as a cheaper alternative procedure especially in areas where anesthetics are not readily available [6,7].

Materials and Methods

Animals

Eighteen apparently healthy (18) 1-3 years old female Philippine native goats (*Capra hircus*) weighing 10-20 kg, body condition score of 2-3 out of 5, were used in this study. Before the start of the 14 days acclimation period, fecalysis and complete blood count were conducted as a screening test to rule out any underlying problems. All animals were dewormed orally on day 1 of acclimation period. The animals were housed at the Nueva Vizcaya State University Small Ruminant Project housing, Bayombong, Nueva Vizcaya and were subjected to standard feeding and housing management procedures. Packed cell volume and white blood cell count were determined a day before the scheduled surgery to ensure that the animals were fit to undergo surgery. The 18 animals were randomly distributed into three groups with six animals per group using a computer generated randomization list in Microsoft Excel® as follows:

- Distal paravertebral nerve block (DPNB);
- Conventional needle acupuncture (CNA); and
- Aquapuncture using 2% chili pepper decoction (AQP).

Methods

Distal paravertebral nerve block (DPNB)

The animal was restrained in right lateral recumbency and distal paravertebral nerve block was performed according to standard procedures Hendrickson and Baird [8]. The distal ends of the transverse processes of the first, second and fourth lumbar vertebra were identified and used as landmarks to block the dorsal and ventral branches of the 13th thoracic, 1st lumbar and 2nd lumbar spinal nerves. The skin was disinfected and 1-3ml of 1% lidocaine hydrochloride was injected at each point using a 2.5cm 23-gauge needle, depending on the body condition of the animal (Table 1).

Table 1: Surgical analgesia in goats produced through distal paravertebral nerve block (DPNB), conventional needle acupuncture (CNA) and aquapuncture (AQP).

Level of Analgesia	Treatments		
	DPNB N = 6	CNA N = 6	AQP N = 6
Excellent	5/6 (83.3%)	0/6 (0.0%)	4/6 (66.7%)
Good	1/6 (16.7%)	1/6 (16.7%)	1/6 (16.7%)
Fair	0/6 (0.0%)	0/6 (0.0%)	0/6 (0.0%)
Poor	0/6 (0.0%)	0/6 (0.0%)	1/6 (16.7%)

Conventional needle acupuncture (CNA)

The acupoints that were used in this study were selected based on the previous study conducted by Acorda [5] in sheep. In their study, these acupuncture points provided a wide area of analgesia on the flank region through pin pick and pinch probe methods but this has not been confirmed by actual surgery. The acupoints used were as follows:

- Acupoint BA04 (Yao-bu zhue-pang) is bilateral and located 3 cm lateral to the depression between the spinous processes of the 13th thoracic and 1st lumbar vertebra, and between the 3rd and 4th lumbar vertebra.
- Acupoint 31 (Bai-hui, Hundred meetings) is located on the dorsal midline between the spinous processes of the 6th lumbar and 1st sacral vertebra
- Acupoint 22 (Qi-jia) is located on the depression between the spinous processes of the 4th and 5th thoracic vertebra.
- Acupoint 26 (Tian-ping, Heavenly Peace) is located on the dorsal midline between the spinous processes of the 13th thoracic and 1st lumbar vertebra.
- Acupoint 47 (Wei-gen) is located at the dorsal midline of the mobile intervertebral joint of the tail base.

After thorough disinfection of the acupoints, a 2.5-cm long, 0.65 cm in diameter straight round sharp filiform conventional acupuncture needle was inserted in the acupoint. The needle was inserted perpendicularly 1-2 cm deep and was stimulated by continuous thrusting and twirling movement of the needle

for one minute. Stimulation of the acupoints was repeated 5min intervals for 30min.

Aquapuncture using chili pepper decoction (AQP)

A 2%percent chili pepper decoction was prepared by boiling 5 g chopped chili pepper (*Capsicum frutescens*) in 500ml water for 30min. The decoction was allowed to cool down, strained and filtered using a filter paper. After the final filtration, sufficient amount of distilled water was added to the solution to bring the decoction to 250ml. The same acupuncture points used in CAN were used for aquapuncture. One ml of chili pepper decoction was injected in every acupoint with a depth of 1-2cm using a disposable 2.5cm 23-gauge hypodermic needle. The needle was withdrawn immediately after injection (Table 2).

Table 2: Mean (± SD) respiratory rates (breaths/min), heart rates (beats/min) and rectal temperature (°C) before, during and after exploratory laparotomy in goats distal paravertebral nerve block (DPNB), conventional needle acupuncture (CNA) and aquapuncture (AQP).

Treatment			
Time	DPNB	CNA	AQP
Respiratory Rate			
Before surgery	18.17 + 2.56 ^a	17.00 + 1.41 ^a	21.17 + 2.48 ^b
During surgery	15.67 + 1.37 ^a	15.33 + 1.03 ^a	19.67 + 3.67 ^b
After surgery	16.67 + 2.80 ^a	15.50 + 1.64 ^a	16.67 + 2.42 ^a
Heart Rate			
Before surgery	83.00 + 8.83 ^a	75.83 + 22.46 ^a	108.33 + 35.21 ^b
During surgery	77.67 + 17.55 ^a	87.17 + 18.13 ^a	116.00 + 20.24 ^b
After surgery	77.67 + 17.91 ^a	89.67 + 15.71 ^a	84.83 + 38.76 ^a
Rectal Temperature			
Before surgery	37.25 + 0.29 ^a	37.75 + 0.85 ^a	37.77 + 1.01 ^a
During surgery	37.10 + 0.48 ^a	37.62 + 1.05 ^a	38.03 + 0.57 ^a
After surgery	37.62 + 0.62 ^a	37.92 + 0.93 ^a	38.80 + 0.51 ^b

Means with different superscripts in the same row are different (P<0.05).

Determination of analgesia and physiological responses

The level of surgical analgesia was determined by using pin prick and pinch probe methods. The pain was graded as excellent, good, fair or poor based on the criteria used by Rogers as follows:

- a. Excellent:** No indication of pain at any stage in the operation. The operation proceeds smoothly at all stages and no other anesthetic method is required. Slight muscle tremor may occur.
- b. Good:** Short intervals of restlessness or mild struggling during traction or suturing of sensitive structures. Local muscle tremor is noted at intervals but the operation can be accomplished without other anesthetics.

c. Fair: Some pain reactions are noted at intervals during the operation and frequent, intermittent struggling may arise. Marked local tremor may also arise. The operation can still proceed. Some of the effects may be relieved by stimulating the embedded needles.

d. Poor: Intense pain response (struggling, vocalization, muscle tremor, etc.) occurs, the operation cannot be carried out without other form of anesthesia.

Respiratory rate, heart rate and rectal temperature were taken before the induction of anesthesia and every five minutes thereafter until the completion of surgery. Respiratory rate was recorded by observing the upward and downward movement of the thoracic cage. The heart rate was obtained using a stethoscope placed on the left thorax caudal to the elbow joint. Rectal temperature was determined using a digital thermometer placed inside the rectum.

Determination of cortisol level

Three milliliters of blood were collected from the jugular vein of each animal before placement of needles on the acupoints for both CNA and AQP and before placement of local anesthetic for DPNB (before), 5 min after peritoneal incision (during) and after the placement of the last skin suture and removal of acupuncture needle (after). The serum was collected and serum cortisol concentration was determined using Cortisol Microplate EIA Test System by Diagnostic Automation, Inc. (USA) as follows. Before proceeding with the assay, all the reagents, serum references and controls were brought to room temperature (20-21 °C). The microplate wells were formatted for each serum reference, control and patient specimen to be assayed in duplicate. Twenty-five microliters of serum reference, control or specimen were pipette and placed into the assigned wells. A 50 µ l of the working Cortisol Enzyme Reagent were added to the all the wells and the microplate are swirled gently for 20-30 seconds to mix. After mixing, a 50 µ l of Cortisol Biotin Reagent were added to the wells and swirled gently for 20-30 seconds to mix. The microplates were covered and incubated for 60min at room temperature. After the incubation period, the contents of the microplate were discarded by decantation or aspiration and the plates were blotted dry with absorbent paper after decantation. A 350 µ l of wash buffer was added to the wells, decanted or aspirated. This procedure was repeated two additional times for a total of three washes. One hundred microliters of working substrate solutions was then added to all wells. To minimize reaction time difference between wells, the reagents were added in the same order. The microplates were incubated at room temperature for 15min. Stop solution (50 µ l) was then added to each wells and gently mixed for 15-20sec. The absorbance in each well was read at 450nm in a microplate reader.

Exploratory laparotomy

Animals were randomly scheduled for surgery between 8:00 in the morning until 6:00 in the afternoon. Goat scheduled

for surgery was fasted for 12 hours but water was given until 6 hours before surgery. The animal was prepared by clipping the hair a day before the scheduled surgery. Analgesia was induced either through DPNB, CNA or AQP with the animal tied and cast down on right lateral recumbency. Exploratory laparotomy was performed according to standard procedures Hendrickson, Baird [8]. After thorough exploration; the abdominal incision was closed in two layers using 3-0 catgut in a simple continuous suture pattern: peritoneum and transverse abdominal muscle, internal and external abdominal muscles. The skin was then sutured using continuous Ford interlocking pattern with 3-0 silk. A light gauze bandage was placed to cover the wound and was removed after 24 hours. The wound was checked every other day for any dehiscence, occurrence of wound infection and suture breakage. Long acting oxytetracycline antibiotic (7mg/kg) was administered for seven days intramuscularly in all treatment groups. Skin suture was removed after seven days (Table 3).

Table 3: Mean (\pm SD) cortisol levels (ng/ml) before, during and after exploratory laparotomy of goats distal paravertebral nerve block (DPNB), conventional needle acupuncture (CNA) and aquapuncture (AQP).

Time	Treatment		
	DPNB	CNA	AQP
Before Surgery	573.62 + 219.72 ^a	605.05 + 381.05 ^a	549.05 + 342.84 ^a
During Surgery	860.57 + 118.89 ^a	872.00	854.22 + 138.38 ^a
After Surgery	861.55 + 173.39 ^a	857.12 + 178.94 ^a	773.40 + 200.64 ^a

Means with different superscripts in the same row are different ($P < 0.05$).

Analysis

The level of analgesia was analyzed through Friedman's two way non-parametric ANOVA. Cortisol level, respiratory rate, heart rate and rectal temperature were analyzed thru PROC GLM for analysis of variance at $P < 0.05$ and Scheffe test for treatment comparison. The protocol used in the study has been approved by the Institutional Animal Care and Use Committee of the College of Veterinary Medicine, University of the Philippines Los Baños.

Results and Discussion

Surgical analgesia

Out of 18 experimental animals, only 12 animals, six for DPNB, five for AQP and one for CNA, achieved at least a satisfactory level of analgesia for exploratory laparotomy (Table 1) during the different periods of observation. No significant linear relationship between treatment and analgesia ($P = 0.5174$) and between period of observation and analgesia ($P = 0.0575$) was observed. For the DPNB group, six goats underwent exploratory laparotomy. Analgesia was achieved within 5-8 min after deposition of the local anesthetic. This is within the

range of 5-20 min for the nerve block to be fully effective Allen and Borkowski [9]. Five out of six showed an excellent level of analgesia before surgery until the end of surgery. Lidocaine, a commonly used local anesthetic, prevents nerve depolarization and conduction of nerve impulses by blocking sodium channels Cornick-Seahorn [10]. It is a short acting local anesthetic due to its low protein binding at the receptor but a faster onset of action owing to its dissociation constant (pKA) closer to plasma pH Grimm 2015. Local anesthetics are commonly used for surface anesthesia, local infiltration, intravenous regional anesthesia, and nerve and spinal block Allen and Borkowski [9].

For the CNA group, only one of the six goats achieved a sufficient level of analgesia for exploratory laparotomy. In most studies, it takes about 15-30-min of stimulation of the acupuncture points to achieve a good result. But since there was already a satisfactory level of analgesia even only after 5 min of stimulation, exploratory laparotomy was performed. The insufficient level of analgesia produced in the five other animals of CNA may be attributed to the poor weather condition, cloudy sky with light rains, and low environmental temperature of 16.5-20.0 °C during the 1st and 3rd day of surgery. The good level of analgesia produced in one animal was observed on the 2nd day of surgery when the weather improved, the sun started to shine and the temperature increased to 25 °C. This finding agrees with the observation of Acorda [5] & Acorda et al. [11] who observed that sheep subjected to conventional needle acupuncture during rainy weather did not exhibit analgesia.

For the AQP group, four goats showed an excellent level of analgesia while one goat exhibited a good level of analgesia before surgery until the end of surgery. Exploratory laparotomy was adequately performed in the five animals. Poor analgesia was observed in one goat after stimulation of the acupuncture points; thus, exploratory laparotomy was not conducted. After deposition of the aquapuncture solution into the acupoints, analgesia was achieved within 5-6 min which was shorter compared to the study by Lamotte [12] requiring 15 min for intradermal injection of capsaicin to induce hyposensitivity to pin prick method or in the studies of Acorda [5] & Acorda et al. [11] which required 10-20min to induce aquapuncture analgesia.

Injection of capsaicin causes burning sensation and hyperalgesia to both thermal and mechanical stimuli surrounding the injection site. Such sensations disappear within 1-2 hours LaMotte [12]. Pain during deposition of chili pepper decoction at the acupuncture points were observed in the goats under AQP group as demonstrated by increased vocalization and struggling. However, hyperalgesia to stimuli was not observed probably due to the exposure to cold environmental temperature. Chung and Wang [13] stated that cold temperature suppressed the activation of TRPV1. This is exemplified by cold therapy to reduce pain following acute injury or surgery. The same findings were observed by Knolle et al. [14]. Cooling the application sites for 8% capsaicin patch to 20 °C prior to application causes a significant reduction in pain induced by capsaicin compared

to non-cooled sites. In the AQP group, one goat did not attain a sufficient level of analgesia which may be due to differences in animal sensitivity to capsaicin Conn [15].

Physiological responses

Respiratory rate: From the initial (before) value, a minimal decrease in the respiratory rate was observed during exploratory laparotomy in all treatment groups (Table 2). A slight increase in the value was observed in DPNB and CNA after exploratory laparotomy. AQP showed a continuous decrease in the value of respiratory rate from the initial (before) value until the completion (after) of exploratory laparotomy. Analysis of data showed significantly higher respiratory rate before and during surgery in AQP but not after surgery compared to the other groups. The respiratory rate in all treatment groups from the initial (before) until the end (after) of surgery were within the normal range (10-30 breaths/minute). This is probably due to exposure to low environmental temperature when the animals inhale cold air, leading to decrease baseline ventilation and respiratory chemo sensitivity. This response mechanism of the body provides a protection against heat loss. The same finding was observed by Diesel et al. that as ambient temperature decreases, respiratory frequency also decreases.

In this study, the use of Lidocaine on DPNB group caused an insignificant change in the respiratory rate. Lidocaine has no effect on the respiratory rate and heart rate DeRossi et al. [16] which is similar to the findings on the study conducted by Atiba et al. [17] in buffalo calves under caudal epidural, Dehkordi in goats under epidural analgesia, Olaifa [18] in goats under distal par vertebral nerve block. Acorda [5] & Acorda et al. [11] observed no changes in the respiratory rate of sheep before, during and after application of acupuncture analgesia.

Heart rate: The mean (+SD) heart rate is presented in Table 2. In DPNB group, the heart rate (HR) was within the normal range before the surgery (70-95 beats/minute) Lin and Walz [19]. During surgery, it decreased from the initial value (before) then remained unchanged after surgery. In contrast, CNA group showed an increased in the heart rate during and after surgery compared to the value before surgery. For AQP, there was a slight increase during surgery and radical decrease after surgery compared to the value before surgery. Data analysis showed that AQP had a significant higher heart rate before and during surgery compared to the other treatment groups. Administration of capsaicin produces complex or mixed effects (bradycardia or tachycardia) on the cardiovascular system Iversen [20]. Chahl, Lynch [21] even stated a triphasic effect on blood pressure after intravenous injection of capsaicin in rats. The initial effect of capsaicin is a decrease in blood pressure and heart rate followed by a temporary and then sustained increase in blood pressure. This can be supported by several studies that capsaicin increases heart rate in rat Ohnuki. Acorda [5] & Acorda et al. [11] observed no changes in the heart rate of sheep before, during and after application of acupuncture analgesia.

Rectal temperature: As shown in Table 2, the rectal temperature in DPNB and CNA showed an increase during and after exploratory laparotomy when compared to the initial (before). When comparing the value from the initial (before) rectal temperature in AQP, there was a decrease during exploratory laparotomy and increase after surgery. Most of the values recorded (77.78%) from the initial (before) until the end of surgery in all treatment groups were below the normal resting rectal temperature (38.7-40.7 °C) (FAO) indicating hypothermia. In AQP, the value recorded after exploratory laparotomy was within the normal range. No significant changes in rectal temperature were observed before, during and after surgery in DPNB and CNA groups. Acorda [5] & Acorda et al. [11] observed no changes in the rectal temperature of sheep before, during and after application of acupuncture analgesia. Data analysis revealed a significantly higher rectal temperature after surgery AQP compared to the other groups. This can be attributed to capsaicin's effect on body temperature by simultaneously stimulating the forebrain heat loss mechanism and the brain stem heat production. It causes transient heat loss and a long lasting heat production Lee [22]. Capsaicin stimulates cutaneous warm receptors and polymodal pain receptors but has no effect on cold receptors or mechanoreceptors. This disturbance on temperature regulation can lasts for months and is characterized by a normal body temperature at cool environment.

Cortisol level

From the initial (before) cortisol level, DPNB showed an increase in the value during and after exploratory laparotomy (Table 3). A decrease in the cortisol level was observed in CNA (one animal) after surgery compared to the value during exploratory laparotomy. In AQP, cortisol level decreased significantly after surgery compared to the level during surgery. Data analysis showed no significant differences in the level of cortisol among the three treatment groups before, during and after exploratory laparotomy. In this study, the mean cortisol value in all treatment groups was higher than the normal value of 65+8nmol/L [23]. This can be due to prior exposure to stressors before exploratory laparotomy. The acclimation period was conducted during the months of December and January, considered as the coldest months of the year, with continuous drizzling and temperature of 16.5-25.0 °C. Exposure of the animals to this very low environmental temperature is one of the possible main stressor, thus producing a very high cortisol level. Such findings coincide with the result of the study conducted by Mellado [24]. In horses, acupuncture analgesia increased cortisol level [25] Acupuncture treatment also increased cortisol level among humans [22].

Other possible stressors that can cause acute stress response may include separation from pen mates prior to surgery due to randomization, restraining, repeated blood collections, exposure to new handlers, exposure to unfamiliar surroundings and the surgical operation. Fear due to isolation or exposure to new individuals causes stress to the animal and consequently an increase in the cortisol level Mober, Mench [26] & Fazio [27].

The animals were accustomed to only two handlers during the acclimation period. When they were brought for surgical operation, they were brought in unfamiliar surroundings and in contact with more new individuals. Restraining the animal during preparation and blood collection for cortisol assay is a form of stress, thus increasing the cortisol level Hopster [28] & Kruger [29]. According to Desborough, plasma concentration of both adrenocorticotrophic hormone and cortisol increases within minutes at the start of surgery.

The results of the study suggest that surgical analgesia produced by aquapuncture using 2% chili pepper decoction is comparable to that produced by distal paravertebral nerve block using lidocaine hydrochloride. The use of chili pepper decoction for acupuncture has several advantages over the use of local anesthetic. Chili pepper decoction poses no toxic effect which is very important in high risk patients. The induction time is also the same with that of local anesthetic [29-42], and it even causes an increase in physiological responses which are very advantageous when the animals are exposed to cold environment before surgery. Thus, aquapuncture can be considered as a cheaper alternative to regional analgesia for production of surgical analgesia in goats. It is recommended that the use of aquapuncture using chili pepper decoction for production of analgesia in other surgical procedures in goats and in other ruminants be investigated. Studies to explore the effect of the environment (weather, temperature, humidity, altitude, etc.) on acupuncture can also be conducted. Furthermore, since aquapuncture has a potential for use especially in areas with limited access to expensive anesthetics, studies on other plant materials available in the local areas for use in aquapuncture analgesia are recommended.

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