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Full Length Article

Serum cortisol of Sahel goats following rumenotomy with assorted anaesthetics and sutures

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Abstract The utmost need for pragmatic combination of surgical sutures and local anaesthetic that would evoke minimal post-surgical stress response and allow uncomplicated healing is essential for successful surgeries. Fifteen Sahel goats were randomly allocated into three groups A, B and C to quantitatively assay (ELISA) serum cortisol profiles following rumenotomy, as markers of surgical stress. Diazepam at 0.2 mg/kg was administered intravenously to groups A and B with subsequent lidocaine HCl and bupivacaine inverted-L block respectively. Group C did not receive any treatment. Chromic catgut (CCG) and polyglycolic acid (PGA) sutures were used for rumen and abdominal muscles closure for groups A and B respectively and nylon for skin closure. Blood samples were taken at post anaesthetic induction (PAI) and post-surgery at 0, 5, 8, 24, 48 and 72 h. The Group A goats expressed serum cortisol that was significantly high 52.76 ± 6.12 ng/mL at 5 h post-surgery. At 8 h post-surgery serum cortisol for both groups A (72.53 ± 3.79 ng/mL) and B (61.59 ± 3.90 ng/mL) were at their peak. Serum cortisol levels compared to the baseline data were significantly different ($P < 0.05$) at 5, 24, and 48 h for the CCG goats. The serum cortisol levels at 72 h drastically decreased to 20.53 ± 8.74 ng/mL for groups A and 17.59 ± 2.45 ng/mL for group B and were not significantly different ($p > 0.05$). Cortisol responses unambiguously indicate that diazepam-bupivacaine induce less stress than Diazepam-lidocaine, hence a preferred anesthesia. Moreover, polyglycolic acid sutures are associated with less inflammatory reaction than chromic catgut.

1. Introduction

Cortisol is an important steroid hormone secreted by the adrenal cortex and associated with inflammation, carbohydrate metabolism and stress reaction [1]. Stress is a disruptive event accompanied by predictable biochemical, physiological, cognitive and behavioral changes that are directed either toward altering the stressful event or accommodating to its effects [2]. It refers to external and internal forces (stressors) applied to animals or other biological systems, leading to measurable alteration of a physiological steady state. These changes termed stress response comprise of metabolic, immunological and neuroendocrine changes following injury or trauma [3,4].

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Stressors are classified as physical/environmental or psychologic/perceived [5]. These stressors influence activation of the hypothalamic pituitary adrenal axis followed by synthesis of corticotrophin or adrenocorticotrophic hormone (ACTH) by the anterior pituitary. This stimulates adrenal cortical secretion of glucocorticoids leading to increased circulating concentration of cortisol. Surgery is one of the most potent activators of this process among others such as infection, tissue injury, trauma, neoplastic growth or immunological disorder [3,5]. Psychological and physical factors like anxiety and pain influence the activation of the hypothalamic–pituitary–adrenal axis (HPA) leading to cortisol release; a core mediator of stress response [3,6] which in turn promotes glycogenolysis and gluconeogenesis resulting in hyperglycemia. The stress induced cortisol secretion exists so that the body can handle and recuperate after an injury, physical activity or physiological strain [3]. Animals respond differently to various kinds of stressors. Plasma cortisol is a useful indicator of stress and muscle damage and data are very limited on responses to surgical stress in veterinary medicine [7]. Studies have shown elevated cortisol concentrations above normal 18 ng/mL levels due to isolation of goats from their social group as emotional stress [7]. When social isolation was not combined with holding treatment, the stress levels were significantly lower in goats that were able to maintain visual contact with other animals [7]. Stressors such as transportation significantly increases plasma cortisol levels within 30 min after beginning of transportation and reached a peak value at one hour in adult male goats; in addition, young goats had higher plasma cortisol concentrations than older ones [7]. The stress indicators and markers elucidate the prospective direction of stressors. These aid in selection of anaesthetic agents, sutures and other corrective strategies that will elicit minimum stress and optimum reparative pathways. For instance, local/regional anaesthesia for rumenotomy that helps in reduction of pain and desirable pathophysiological responses [3,8,9], as well as the choice of suture materials that greatly dictates post-surgical tissue reactions and inflammatory processes. Therefore, ''there is a practical need for pragmatic combination of surgical sutures and local anaesthetic that would evoke minimal post-surgical stress response and allow uncomplicated healing''. Sutures and anaesthetic agents putatively produce distinctive tissue reaction and inflammatory process. However, the indicators and biomarkers of surgical stress patterning following surgery in Sahel goats remain unclear. This study aims to evaluate the stress induced by induction of local anesthesia and rumenotomy in the Sahel goat by determining the levels of serum cortisol in Diazepam-Lidocaine Combination (DLC) and Diazepam-Bupivacaine Combinations in the event of choice of chromic catgut (CCG) and polyglycolic acid (PGA) sutures.

2. Materials and methods

2.1. Animals

Fifteen apparently healthy Sahel goats aged 15.10 ± 4.12 months weighing 17.70 ± 3.3 kg bw were procured from Maiduguri livestock market in Borno state, Nigeria. Maiduguri is the Capital of Borno state in the northeastern region of Nigeria. Maiduguri is located within latitude 11.15°N and longitude 30.05°E the Sudano-Savanna zone [10]. Clearance from the Faculty of Veterinary Medicine Animal Ethics Committee, University of Maiduguri, Nigeria was obtained for the research. Using a random number generator (RNG®), the goats were randomly allocated to 3 groups of 5 goats A, B and C, with A and B as experimental groups while C as a control group.

2.2. Preoperative preparation

The goats were fasted for 12 and 6 h for feed and water respectively. The left paralumbar fossa was clipped and the area was aseptically prepared with 0.2% chlorhexidine gluconate (Savlon®, Vervaadingdeur, Johnson and Johnson (pty) Ltd, London) (60 mL stock per liter of sterile water). Prior to anaesthesia and surgery, a drip infusion line was instituted via the jugular vein and commenced fluid therapy with 0.9% normal saline solution (Juhel®, Fabrique par Juhel Nig. Ltd/Awka, Anambra, Nigeria) as maintenance fluid, set at 180 mL per hour (1 drop per second). The experimental goats were sedated with Diazepam, (SJG®, FazulEllahie, Pvt Ltd, Karachi, Pakistan), given at a dose rate of 0.2 mg/kg IV. The goats in group A received 2% Lidocaine HCl 4 mg/kg (Lidocaine®, Kwality Pharmaceuticals (P) Ltd. NagKalan, Majitha Road, Amritsar, INDIA) to create an inverted L-block regional anaesthesia on the left flank (Paralumbar fossa) immediately after sedation. Animals in group B received 0.5% Bupivacaine 1.5 mg/kg (Marcaine®, Astrazeneca PLC, IngiItere Lisansiile, Istanbul, Turkey), for inverted L-block post Diazepam, (SJG®, Fazul Ellahie, Pvt Ltd, Karachi, Pakistan), sedation. Five minutes after induction, 5 mL of blood samples were drawn from the opposite jugular vein into a plain vacutainer tubes and kept at room temperature for 2 h before centrifugation at 4000×*g* for 3 min in an electronic centrifuge (Centrifuge 800B®, Union Laboratories, England).

2.3. Surgery

The standard laparotomy method 'through-and-through' incision was made on the upper paralumbar fossa of the left flank [11]. The rumen was gently pulled out of the incision and firmly anchored to the skin ventrally and dorsally with two of six towel clamps for the technique. A ten centimeter (10 cm) incision was made over less vascular portion of the rumen greater curvature and its edges were fixed caudally and cranially to the skin incision with towel clamps [12]. The remaining clamps were used to secure the rumen edges between the previously placed clamps with their handles away from the incision site. The rumen edges covered the skin edges by 2–3 cm. The rumen were explored and foreign materials mostly plastic bags weighing 1.26 ± 0.18 and 1.44 ± 0.86 kg for groups A and B respectively were removed, 0.9% saline solution was used to rinse the rumen ingesta. To commence rumen closure, the caudal and cranial clamps were removed first leaving the dorsal and ventral clamps in place. Double layer Cushing suture pattern was used to invert the rumen edges with a number 2 chromic catgut (LIFECARE®, Anhui Kangning Industrial group Co. Ltd, Tianchang City, Anhui, China) and polyglycolic acid sutures (Atramat®, Internactional Farmaceutica, Planta, Mexico), for groups A and B respectively.

The skin was closed using a size 2 nylon suture (LIFECARE®, Anhui Kangning Industrial group Co. Ltd, Tianchang City, Anhui, China) for both groups A and B in a Ford interlocking suture pattern [13]. Two surgeries one for each group were conducted daily during the morning hours while group C had no surgery.

2.4. Sample collection

Five milliliter (5 mL) of blood samples were obtained via the jugular veins that were not used for fluid therapy immediately after surgery at 0 h and then 5, 8, 24, 48 and 72 h post-surgery which were emptied into plain vacutainer tubes (Vacuum Tube®, Apex) and allowed to clot at room temperature for 2 h before centrifugation at 4000×*g* for 3 min. Animals in group C were sampled twice, at the commencement of the study and at the end of the study. After centrifugation, harvested sera were emptied into micro-vial and stored at −20 °C until serum cortisol analysis.

2.5. Analysis of serum cortisol by ELISA

The goat CORT ELISA (NeoBiolab®, Serving Science Sharing Science, Cambridge, MA, USA) is a quantitative competitive immunoassay. The microtiter plate provided was coated with a CORT specific antibody. Standards or experimental samples were co-incubated in wells along with an CORT–HRP conjugate. CORT in standards/samples compete with CORT–II–HRP conjugate for binding to the plate bound antibody. Higher levels of CORT from standards or samples lead to decreased CORT–HRP conjugate binding and reduced signal. The captured CORT–II–HRP is quantitatively detected by incubation with HRP substrates (Solutions A and B). Binding of the CORT–HRP is visualized by production of colourimetric reaction products that were quantitatively measured by absorbance at 450 nm using a molecular microplate reader device (E max®, precission microplate reader, USA). The data obtained in this study were analyzed within groups using One Way Repeated Measures ANOVA with a Dunnett's Multiple Comparison Post Test and between groups with Two Way Repeated Measures ANOVA with Bonferroni post test. Column statistics was used to determine M ± SD of the groups. Graphpad Prism Version 4.0, (2003) software was used for the data analysis. Analyses were considered as significant at ($P < 0.05$).

3. Results

Serum cortisol levels at five minute post anaesthetic induction (PAI) and different periods of surgery for Goats in groups A, B and C are presented in (Table 1). The means were significantly different within the groups ($P < 0.05$). The baseline serum concentrations of goats in groups A and B are 16.12 ± 2.69 ng/mL and 14.75 ± 0.83 ng/mL respectively. Serum cortisol levels were maintained at PAI and 0 h post-surgery for group A and up to the 5th hour post-surgery for group B. The Group A goats expressed serum cortisol that was significantly high 52.76 ± 6.12 ng/mL at 5 h post-surgery. At 8 h post-surgery serum cortisol for both groups A (72.53 ± 3.79 ng/mL) and B (61.59 ± 3.90 ng/mL) were at their peak. Serum cortisol levels compared to the baseline data were significantly different ($P < 0.05$) at 5, 24, and 48 h in group A. Serum cortisol level in group B goats was maintained up to the fifth hour post-surgery ($P > 0.05$) before it peaked at 8 h post-surgery. The serum cortisol levels at 72 h drastically decreased for groups A and B, and were not significantly different ($P > 0.05$).

4. Discussion

Neuronal activation of the hypothalamic pituitary adrenal axis (HPA-axis) comprises of hormonal changes which can be moderated by anaesthesia. These changes when not moderated are responsible for stress response after surgery. Studies have shown normal goat cortisol concentrations as 14–18 ng/mL [7]. The serum cortisol levels of goats in groups A and B were steadily maintained up to 2 h and 5–6 h respectively. These findings may suggest that local anaesthetics in inverted-L block delay cortisol production since afferent nerve input from the operative site to the central nervous system and the hypothalamic pituitary axis (HPA) are blocked. The blockade of efferent neuronal pathways to the liver and adrenal medulla produces the same effect [3,6]. Cortisol response was earlier (5 h) in group A while in group B it was noted at 8 h which suggests that these responses are related to the onset and duration of action of the local anaesthetics. The peak cortisol levels were seen at 8 h post-surgery for both CCG and PGA groups and began to fall at 24 h for both groups but were still significantly different until 72 h when both levels declined to normal range ($P > 0.05$). Synthesis of cortisol was inhibited by the local anaesthetics and benzodiazepines [3,6]. Diazepam

Table 1 Mean values of goat serum cortisol post anesthetic induction (PAI) and at different periods post-surgery in all groups.

Group	Baseline	PAI (5 mins)	M ± SD of serum cortisol values (ng/mL) at various periods post-surgery					
			0 h	5 h	8 h	24 h	48h	72h
A	16.12 ± 2.69[a]	15.65 ± 1.18[a]	16.55 ± 1.88[a]	52.76 ± 6.12[b]	72.53 ± 3.79[c]	61.71 ± 5.06[d]	37.10 ± 14.21[e]	20.53 ± 8.74[a]
B	14.75 ± 0.83[a]	14.18 ± 0.15[a]	14.51 ± 0.48[a]	14.95 ± 1.63[a]	61.59 ± 3.90[b]	43.44 ± 9.64[c]	17.44 ± 3.46[a]	17.59 ± 2.45[a]
C	16.51 ± 2.99[a]	–	–	–	–	–	–	15.64 ± 0.83[a]

Values with different superscripts within a row are significantly different ($P < 0.05$).
Goats in group A and B had Diazepam-lidocaine with chromic catgut sutures and Diazepam-bupivacaine with PGA sutures respectively for anaesthesia and rumen and abdominal muscle closure.

acts on the hypothalamic pituitary level to produce a direct inhibitory effect on steroid production [3]. The findings in this study suggest that inverted-L block in goats produced similar responses. Epidural blockade from dermatomal segment T4 to S5 prevented increase in cortisol because both afferent input from the operative site to the CNS and HPA-axis, and efferent autonomic neuronal pathways to the liver and adrenal medulla were blocked [14].

5. Conclusion

The Cortisol responses unambiguously indicate that diazepam-bupivacaine induce less stress than Diazepam-lidocaine, hence a preferred anesthesia. Moreover, polyglycolic acid sutures are associated with less inflammatory reaction than chromic catgut.

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References

[1] Sjaastad OV, Hove K, Sand O. Physiology of domestic animals. Scandinavia: Scandinavian Veterinary Press; 2003. p. 223–4.
[2] Taylor SE. Health Psychol. 6th ed. Washington: McGraw-Hill Humanities; 2008. p. 3–9.
[3] Desborough JP. The stress response to surgery. Br J Anaesth 2000;85:109–17.
[4] Badyaev AV. Stress-induced variation in evolution: from behavioural plasticity to genetic assimilation. Proc Roy Soc B 2005;272:877–86.
[5] Gruys E, Toussaint MJM, Landman WJM, Tivapasi M, Chamanza R, Veen LV. Infection, inflammation and stress inhibit growth. mechanisms and non-specific assessment of the processes by acute phase proteins. In: Production diseases in farm animals 10th international conference, 1998. Wageningen: Wageningen Press; 1999. p. 72–87.
[6] Burton D, Grainne N, George H. Endocrine and metabolic response to surgery. Contin Educ Anaesth Crit Care Pain 2004;4 (5):144–7 .
[7] Kannan G, Terrill TH, Kouakou B, Gazal OS, Gelaye S, Amoah EA, et al. Transportation of goats: effects on physiological stress responses and live weight loss. J Anim Sci 2000;78:1450–7.
[8] Oguntoye CO, Adetunji A. Evaluation of lignocaine/bupivacaine mixture for central neural blockade in goats. Isr J Vet Med 2009;64(4):122–7.
[9] Olaifa AK, Olatunji-Akioye AO, Agbaje LO. Distal paravertebral nerve block effects on west african dwarf goat haematology and physiology. Isr J Vet Med 2009;64(4):128–31.
[10] Udo RK. A comprehensive geography of West Africa 1978:4–18.
[11] Firth EGD, Fontijne P, Kersjes AW, Nemeth F, Rutgers LJE, Van der Velden MA, editors. Atlas of large animal surgery. Utretcht, The Netherlands: Koninklijke Smeets Offset BV, Weert; 1985. p. 39–42.
[12] Dehghani SN, Ghadrdani AM. Bovine rumenotomy: comparison of four surgical techniques. Can Vet J 1995;36:693–7.
[13] Weaver D, Steiner A, Jean GS. Bovine surgery and lameness. Garsington, UK: Blackwell Publishing Ltd; 2005. p. 45–90.
[14] Manorama S. Stress response and anaesthesia. Indian J Anaesth 2003;47(6):427–34.