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Periodic Indices of Serum Amyloid A Following Rumenotomy with Assorted Local Anaesthetics and Sutures in the Sahel Goat

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Abstract

Objective- This study aims at validating the use of serum amyloid A (SAA) as biomarkers of surgical stress using Quantitative ELISA in the Sahel goats. The experiment evaluated SAA profiles of Sahel goats post Diazepam-Lidocaine (DLC) and Diazepam-Bupivacaine (DBC) local anaesthesia. Expressions of SAA in the Sahel goat post rumen skin clamp fixation techniques of rumenotomy with Polyglycolic acid sutures (PGA) and chromic catgut sutures were also expounded.
Design- Experimental Study.
Animals- Fifteen Sahel goats were randomly allocated into three groups A, B and C for quantitative ELISA evaluation of Serum Amyloid A (SAA) profiles post local anaesthesia and laparo-rumenotomy, as biomarkers of surgical stress.
Procedures- Diazepam at 0.2mg/kg was administered intravenously to sedate goats in groups A and B with subsequent lidocaine HCl and bupivacaine inverted-L block anaesthesia respectively. Chromic catgut (CCG) and Polyglycolic Acid (PGA) sutures were used for rumen and abdominal muscle closures for groups A and B respectively while nylon for skin apposition. Blood samples were collected via jugular venipuncture to establish baseline data after local anaesthesia (PAI) and at 0, 5, 8, 24, 48 and 72 hours after surgery.
Results- Group A peak SAA values was 9.29±0.43 µg/mL at 8hrs post-surgery while goats in group B had a peak of 10.94±1.22 µg/mL SAA at 24hrs post-surgery. SAA responses of group A indicates stress at 0hr steadily throughout the first 72 hours but peaked at 8hrs post-surgery. Similarly, group B showed significant stress at 8 hours onwards with peak values at 24 and 48 hours post-surgery.
Conclusion and Clinical Relevance- SAA as an acute phase protein could be used to determine early complications following laparo-rumenotomy in the goat and offers good prognosis especially when several APP variables are combined in an index.
Keywords- Serum amyloid A, Goats, Sutures, Rumenotomy, Anaesthetics

Introduction

Acute phase proteins (APPs) are blood (serum or plasma) molecules synthesized by many cell categories, especially hepatocytes that change in concentration in animals subjected to external or internal impetus or alterations such as infection, inflammation, surgical trauma, or stress.[1-3] Acute phase protein (APP) profiles of all species varies and as such must be examined individually since APP analysis is becoming a common procedure in clinical and experimental investigations of infectious disease in farm and companion animals.[4]

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Serum amyloid A (SAA) are acute-phase apolipo-proteins (non-glycosylated) connected to high-density lipoprotein (HDL) in inflammation with a molecular weight ranging between 11 and 14 kilodaltons (KDa), depending on species. Serum amyloid A is so named due to its involvement in reactive amyloidosis.[5] The complete biological function of this protein is not yet fully expounded, but three different functions: binding of cholesterol, immunomodulatory activity and opsonisation have been attributed to this protein.[6] Investigations over the last decade have shown that the quantification of their concentration in plasma or serum can provide valuable diagnostic information in the detection, prognosis and monitoring of disease.[4] The normal range of SAA in goats is 0.42-2.2µg/mL but varies decidedly among species.[7] Diazepam is the second invented benzodiazepine which has a CNS depressant effect by slowing normal brain function.

Diazepam 0.1- 0.25 mg/kg has been found useful in many species, particularly in goats. It is given slowly intravenously and provides 30 minutes of good sedation. The animal can be quite aroused and there is no analgesia.[8,9] Goats are ideally suited to local anaesthetic techniques under sedation or manual restraint. Lidocaine is the most commonly used of the local anaesthetic solutions and is well tolerated by goats.[10] Inverted L block is an easy technique of local or regional anaesthesia. Lidocaine possesses reasonably rapid onset of action, with good (3-5 minutes, short duration of action (60-75 minutes), with good spreading properties, being a good 'all round' useful local anaesthetic. Bupivacaine is an amide with long acting properties up to eight hours when combined with epinephrine that is appropriate for procedures that last 2-2.5 hours. It has a slow onset of action ranging from 5-10 minutes after induction. It is therefore used whenever long action is required for post-operative analgesia or prolonged surgery.[10] The major advantages of bupivacaine with particular emphasis on clinical use in surgery compared with other presently used local anaesthetics are an increased duration of action and a favorable potency to toxicity ratio. As sutures and anaesthetic agents putatively produce distinctive tissue reaction and inflammatory processes, indicators and biomarkers of surgical stress patterning following surgery in the Sahel goats remain unclear. This study evaluates stress induced by diazepam sedation and local anaesthesia and surgery by providing SAA profiling following laparo-rumenotomy in different choice of local anaesthetics and suture materials in Sahel goats.

Materials and Methods

Experimental Design

The work was designed to provide serum amyloid A (SAA) expressions of Sahel goat post rumenotomy with Diazepam-Bupivacaine anaesthesia using Polyglycolic acid sutures (PGA) as well as in Diazepam-Lidocaine anaesthesia using chromic catgut sutures.

Study Area

Fifteen apparently healthy Sahel goats aged 15.10±4.12 months weighing 17.70±3.3 kgbw were procured from Maiduguri livestock market in Borno state, Nigeria. Maiduguri is the Capital city of Borno state in the northeastern region of Nigeria. Maiduguri is located within latitude 11.15^0N and longitude 30.05^0E the Sudano-Savanna zone.[11]

Animals

The animals were kept in the Department of Veterinary Surgery and Radiology pens following due clearance from the Faculty of Veterinary Medicine Postgraduate Committee, University of Maiduguri, Nigeria. The goats were fed groundnut husk, concentrates and water was provided *ad libitum*. Animals were allowed to acclimatize for a period of two months before commencement of the experiment and special attention was given to their management. Using a random number generator (RNG®), the goats were randomly allocated to 3 groups of five goats each A, B and C, with A and B as experimental groups while C as control. Blood samples (5mls) each were collected from jugular venipuncture from all the 15 goats to establish base line data before commencement of experiment.

Pre-operative Preparation

The goats were fasted for 12 and 6 hours for feed and water respectively. The animals were placed on right lateral recumbency and the left paralumbar fossa was shaved. The shaved area was aseptically prepared with 4% Chlorhexidine gluconate (Savlon®, Vervaading deur, Johnson and Johnson (pty) Ltd, London) (60ml stock per liter of sterile water). Prior to local 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). Diazepam (SJG®, Fazul Ellahie, Pvt Ltd, Karachi, Pakistan), at a dose rate of 0.2mg/kg IV was used to sedate the animals. Goats in group A received 2% Lidocaine HCl 4mg/kg (Lidocaine®, Kwality Pharmaceuticals (P) Ltd.Nag Kalan, 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 1.5mg/kg (Marcaine®, Astrazeneca PLC, Ingi Itere Lisansi ile, Istanbul, Turkey), for inverted L-block post Diazepam sedation.

Surgery

A standard method of laparotomy, 'through-and-through' incision was made on the center of the upper paralumbar fossa of the Left flank between the last rib and the tuba coxae.[12] A rumen skin clamp fixation technique were performed according to standard.[13, 14] 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 and cleaned. 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 (Weaver *et al.*, 2005) in a Ford interlocking suture pattern. As group C had no surgery, two surgeries comprising one animal from each group were performed daily and all the surgeries were concluded on the fifth day among the experimental groups.

Blood Sample Collection and Serum Processing

Five milliliter (5mL) of blood were drawn via the jugular vein to establish baseline data before commencement of surgery. Five minutes post anaesthetic induction (PAI), 5mL of blood were drawn from the opposite jugular vein into a plain vacutainer tubes and kept at room temperature for 2 hours in an electronic centrifuge (Centrifuge 800B® , Union Laboratories, England). Immediately after surgery, five milliliter (5mL) of blood were obtained via the jugular veins that were not used for fluid therapy at 0 hour and then 5, 8, 24, 48 and 72 hours post-surgery for both groups A and B which were emptied into plain vacutainer tubes (Vacuum Tube®, Apex) and allowed to clot at room temperature for 2 hours before centrifugation, harvested sera were emptied into micro-vial and stored at -20^{o}C until SAA analysis.

G*oat Serum Amyloid A ELISA*

The goat SAA ELISA (NeoBiolab®, Serving Science Sharing Science, Cambridge, Massachusetts, USA) is a quantitative competitive immunoassay. The microtiter plate provided was coated with an SAA specific antibody. Standards or experimental samples were co-incubated in wells along with an SAA– HRP conjugate. SAA in standards/samples compete with SAA-II- HRP conjugate for binding to the plate bound antibody. Higher levels of SAA from standards or samples lead to decreased SAA-II-HRP conjugate binding and reduced signal. The captured SAA-II- HRP is quantitatively detected by incubation with HRP substrates (Solution A and B). Binding of the SAA-II-HRP is visualized by production of colourimetric reaction products that were quantitatively measured by absorbance at 450nm using a molecular microplate reader device (E max®, precission microplate reader, USA).

Statistical Analysis

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 posttest. 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 $P<0.05$.

Results

Rumen skin clamp fixation technique of rumenotomy was performed in ten Sahel goats among fifteen goats used for the study. All the animals in groups A and B survived the procedure without apparent complications throughout the periods of sample collection and two weeks of post-operative care. The skin wounds in group B resolved earlier on gross observation. Stitch reaction was mild in group B animals while it was moderate in group A on gross examination of inflammatory signs. The baseline serum concentrations of SAA in this study was 2.36±0.90 µg/mL for group A and 2.11±0.74 µg/mL for group B. The SAA assay in groups A and B yielded values that were significantly different ($p< 0.05$) compared to the baseline data.

Table 1. Mean Values of Serum Amyloid A (SAA) (µg/mL) after lidocaine and bupivacaine local anaesthesia and at different periods of time after surgery.

Group	Baseline	PAI (5mins)	0hr	5hrs	8hrs	24hrs	48hrs	72hrs
A	2.36±0.90^{a}	2.31±0.63^{a}	4.31±0.65^{b}	8.14±1.63^{c}	9.29±0.43^{d}	7.37±0.81^{e}	7.33±0.83^{e}	8.18±1.65^{c}
B	2.11±0.74^{a}	2.47±0.94^{a}	2.34±0.98^{a}	2.18±0.83^{a}	8.45±0.92^{b}	10.94±1.22^{c}	10.92±1.54^{c}	8.54±1.33^{b}
C	2.21±0.32^{a}	-	-	-	-	-	-	2.41±0.35^{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.
PAI: Post anaesthetic induction

Significant difference ($p< 0.05$) was observed in group A, as SAA levels rose steadily through time from 0, 5, 8, 24, 48, and 72 hours at these concentrations 4.31±0.65 µg/mL, 8.14±1.63 µg/mL, 9.29±0.43 µg/mL, 7.37±0.81 µg/mL, 7.37±0.81 µg/mL, 7.33±0.83 µg/mL, and 8.18±1.65 µg/mL respectively (Table 1). In Group B, SAA concentrations also increased with time and were significantly different ($p< 0.05$) at 8, 24, 48 and 72hrs as 8.45±0.92 µg/mL, 10.94±1.22 µg/mL, 10.92±1.54 µg/mL, 8.54±1.33 µg/mL respectively (Table I). The peak SAA were 9.29±0.43 µg/mL at 8hrs for group A and 10.94±1.22 µg/mL at 24hrs post-surgery for group B. SAA values were significantly different ($p<0.05$) at 72 hours for both groups with SAA values being fourfold of the baseline.

Discussion

Animals may be subjected to stylized and/or controlled stress such as that which arises during premedication, anaesthesia and surgery. These are undertaken for the purposes of examinations, diagnosis, and treatment of conditions such as foreign masses in the rumen, relief of dystocia, and hysterectomy. These procedures induce significant stress (surgical trauma) and impacts the stress response pathways of the body. In this study, RSCF technique was employed because it is a fast technique and produces clean wound with less postoperative complications.[14] Serum amyloid A (SAA) become apparent during inflammation as such a useful indicator of inflammatory processes and surgical stress.[2,15,16] In addition to inflammatory conditions, APPs are also released during gestation and other normal physiologic conditions [(17 et)] but only a change of up to 25% in concentration of these proteins are considered acute phase response or reaction (APR).[18] The mechanism of local anaesthetics influence on the acute phase response is uncertain.[19] The normal range of SAA in goats is 0.42-2.2µg/mL.[7] In group A, the SAA concentrations did not change post anaesthetic induction in diazepam-lidocaine-combination (DLC) until averagely 40 minutes later or rather immediately after surgery (0 hours) when the drug has almost completely vanished from the body, this may be due to the local anaesthetics suppressing the pain threshold delaying the Acute phase protein response.[20, 21] The SAA response was delayed for a longer period in the group B goats that received diazepam-bupivacaine anaesthesia (DBC) until 8 hours post-surgery. This may be attributed to the duration of action of bupivacaine since it last for 6-8 hours before it is fully metabolised by the body.[10] The result suggests that analgesia must be provided 40 minutes post-surgery with DLC in the goat since the surgery duration ranged between 35-40 minutes and was the period the SAA values rose steadily. Local anaesthetics may delay or inhibit stress responses including APPs,[21, 22] and greatest with neural blockade and this has shown to have beneficial effects on surgical out-come for the group B goats.[20] Increased levels of SAA were noticed at 5 and 8 hours post-surgery for CCG and PGA goats respectively which were the earliest periods of the local anaesthetics been totally eliminated or metabolized from the body. SAA levels in the DBC/PGA group stayed high but occurred earlier in the DLC/CCG group which is attributed to the level of analgesia provided by these agents. The mechanism for the increase could be attributed to increase in rapid synthesis of sialoproteins and to an increase in globulins released from damaged tissue triggered by surgical injury and the biomaterials used in closing the incisions and also as reported that SAA levels increase rapidly at the beginning of inflammatory response.[20,21,23] Although PGA is a lesser irritant, it however stays longer and higher concentration than CCG induced SAA elevation. The concentrations of SAA in CCG and PGA goats was highest at 8 hours (9.29± 0.43) and 24 (10.94±1.22) hours for groups A and B respectively. The variability in the results among individuals in the PGA group may be the plausible reason for higher values compared to that of the CCG. This is contrary to the findings of [23] that SAA levels increase rapidly at the beginning of inflammatory response. This may suggest that PGA is less irritant as compared to CCG when used in closing wounds but further studies are required to compare these variables. In conclusion, this study has shown that APPs such as SAA are suitable biomarkers for the assessment of post local anaesthetic and laparo-rumenotomy surgery induced stress. SAA are markers of choice in the early periods post-surgery. SAA have proved that CCG sutures induces stress and a less equivalent with the PGA sutures. Evaluating markers of stress especially SAA in the goat post-surgery, offers good prognosis and determine early complications particularly when several APP variables are combined in an index.

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چکیده

شاخص‌های دوره‌ای آمیلوئید A سرمی بدنبال رومنتومی با بی‌حسی‌های موضعی و بخیه‌های گوناگون در بز نژاد ساحل

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هدف- بررسی استفاده از آمیلوئید A سرمی (SAA) به‌عنوان بیومارکرهای استرس جراحی با استفاده از الایزا کمی در بزهای نژاد ساحل. در این مطالعه پروفایل SAA بعد از بی‌حسی موضعی با دیازپام- لیدوکائین و دیازپام- بوپیواکائین ارزیابی شد. بیان SAA در بز ساحل بدنبال تکنیک فیکساسیون پوست شکمبه با گیره در رومنوتومی با بخیه پلی‌گلیکولیک اسید و کات‌گوت کرومیک همچنین توضیح داده شده است.

طرح- مطالعه تجربی.

حیوانات- ۱۵ بز نژاد ساحل به‌صورت اتفاقی در سه گروه برای ارزیابی الایزا کمی پروفایل SAA به‌عنوان بیومارکرهای استرس جراحی بعد از بی‌حسی موضعی و لاپارو-رومینوتومی قرار داده شدند.

روش کار- دیازپام با دز ۰/۲ میلی‌گرم به‌ازای کیلوگرم بصورت داخل رگی برای آرام بخشی بزها در گروه‌های A و B تجویز شد و متعاقبا به ترتیب بی‌حسی L معکوس با استفاده از لیدوکائین HCL و بوپیواکائین برای گروهای A و B استفاده شد. بخیه‌ها با استفاده از کات-گوت کرومیک و پلی‌گلیکولیک اسید برای بستن شکمبه و عضلات شکمی برای گروه‌های A و B به ترتیب مورد استفاده قرار گرفت. نخ نایلون برای بخیه پوست استفاده شد. نمونه‌های خون از طریق ورید وداج به منظور تعیین داده پایه بعد از بی‌حسی موضعی و در زمان‌های صفر، ۵، ۸، ۲۴، ۴۸، ۷۲ ساعت بعد از جراحی جمع‌آوری شد.

نتایج- در گروه A پیک SAA در ۸ ساعت بعد از جراحی برابر با ۹/۲۹±۰/۴۳ میکروگرم به ازای میلی‌تر و در گروه B پیک برابر با ۱۰/۹۴±۱/۲۲ میکروگرم به ازای میلی‌تر در ۲۴ ساعت بعد از جراحی بود. پاسخ SAA در گروه A بیانگر استرس در زمان صفر بطور پیوسته در ۷۲ ساعت اول بود اما پیک پاسخ در ۸ ساعت بعد از جراحی گزارش شد. بطور مشابه، گروه B استرس معنی‌داری را در ۸ ساعت بعد از جراحی با بالاترین مقادیر در ۲۴ و ۴۸ ساعت بعد از جراحی نشان داد.

نتیجه‌گیری و کاربرد بالینی- SAA می‌تواند به‌عنوان یک پروتئین فاز حاد برای تعیین عواقب زودهنگام به‌دنبال لاپارو-رومینوتومی در بز استفاده شود و پیش‌آگهی خوبی را مخصوصا هنگامیکه چندین متغییر APP یکی می‌شوند پیشنهاد می‌کند.

کلمات کلیدی- آملوئید A سرمی، بز، بخیه، رومنوتومی، بی‌حسی.