

Influence of ketamine, thiopental or propofol anaesthesia on acute phase proteins in buffalo calves

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ABSTRACT

The objective of this study was to compare the changes of acute phase proteins after ketamine, thiopental and propofol anaesthesia in buffalo calves. Five calves were anaesthetized three times with ketamine, thiopental or propofol in random order at 3 weeks intervals. Calves were pre-medicated with xylazine and buterphenol. Propofol resulted in an excellent anaesthetic induction (free of excitement), short recovery times and duration of anaesthesia, whereas ketamine and thiopental produced a good and fair quality of induction respectively. Acute phase proteins showed significant increase in haptoglobin and fibrinogen, whereas albumin was significantly decreased in all groups. Haptoglobin returned to normal values 48 hours after anaesthesia with propofol and thiopental.

In conclusion, the anaesthetic quality produced by propofol in buffalo calves was better compared to those of ketamine and thiopental but it has a short

duration of anaesthesia. Propofol and thiopental significantly decreased acute phase proteins due to anti-inflammatory effects.

INTRODUCTION

Ketamine and thiopental are widely used in ruminants to induce and maintain unconsciousness (Singh et al. 2003). Ketamine has been also recommended for balanced anaesthesia and post-operative analgesia in clinical veterinary patients (Sharma et al. 2004). Propofol has been used extensively in dogs, horses, and human being. Previous studies have revealed that propofol has a high volume of distribution, rapid metabolism and rapid clearance when given by repeated dose or continuous intravenous infusion (Aguiar et al. 1993). The rapid induction and short duration of action, with rapid recoveries make propofol potentially useful in calves (Branson and Gross 1994). However, when used alone, propofol is unsatisfactory; consequently, it has been combined with various sedatives and analgesic

drugs to produce adequate surgical conditions (Cullen and Reynoldson , 1993).

Propofol anaesthesia influenced the release of the pro-inflammatory and the anti-inflammatory cytokines, which are potent inducers of the hepatic acute phase proteins (APPs) synthesis (Brand et al. 1997). Alterations in inflammatory cytokines and APPs can cause abnormal regulation of the inflammatory process, which may aggravate postoperative complications (McBride et al. 1996).

The APPs are a group of blood proteins that change in concentration in animals subjected to external or internal challenges, such as infection, inflammation, surgical trauma or stress. They are considered to be non-specific innate immune components involved in the restoration of homeostasis and the restraint of microbial growth before

animals develop acquired immunity to a challenge (Murata et al. 2004). The circulating concentrations of the APPs are related to the severity of the disorder and the extent of tissue damage or stress in the affected animal; quantification of their concentration can therefore provide diagnostic and prognostic information if proper timing of sampling is assured. There is little information about the impact of propofol, thiopental or ketamine anaesthesia on APPs.

Therefore, the aim of the present study was to compare the effects of propofol, thiopental or ketamine anaesthesia on APPs in buffalo calves.

MATERIALS AND METHODS

2.1. Animals

The study was conducted on five healthy male buffalo calves of 13 ± 1.6 months of age and of mean body weight 175 ± 3.2 kg. The animals were housed indoors under uniform management in feeding and watering (according to the protocol of Faculty of Veterinary Medicine, Benha University, Egypt).

Food was withheld for 24 h prior to the experiment, but the animals had free access to water. All animals received humane care and all efforts were made to minimize animal suffering.

2.2. Anaesthetic protocol

The animals received three drugs combinations in a randomized cross-over design with an interval of 3 weeks before re-use. Xylazine (Adwia, Egypt, 0.2 mg/kg, IV), buterphenol (Stadol; Bristol-Myers, Egypt, 0.05 mg/kg, IV), ketamine (Ketalar; Epico, Egypt, 2. mg/kg, IV), thiopental sodium (8 mg/kg, IV) and propofol (Diprivan, Astra Zinica, UK, 3 mg/kg, IV) were used. The drug combinations were as follow: xylazine, buterphenol, and ketamine; xylazine, buterphenol, and thiopental; xylazine, buterphenol, and propofol. Xylazine was injected firstly followed by buterphenol and 2-3 minutes later ketamine, thiopental and propofol according to drug combination. All drugs were given by IV injection over 60 seconds.

2.3. Evaluation of drugs action and monitoring of anaesthesia

The buffalo calves were evaluated at 0 time (pre-injection), 5, 15, 30 and 45 min to estimate the effects of drugs on motor control, response to pin-prick and degree of sedation/anaesthesia. During induction of anaesthesia, the movement of the buffalo calf from standing to a recumbent posture was assisted. No assistance was provided to the calves during recovery from anaesthesia. Superficial and deep pin-pricks were conducted in the head, neck, shoulder region, tail, perineal region, and upper hind limb as noxious stimuli to evaluate the produced analgesia. Response to pin-prick was recorded on score 0–3 (0: Strong; 1: Mild, showing frequent response with pin-prick; 2: Moderate, showing occasional response with pin-prick; 3: Severe, complete loss of response to pin-prick). Induction time, quality of induction and recovery, time of sedation, time of analgesia, heart rate, respiratory rate and the incidence of side effects, e.g. apnea, regurgitation,

hypersalivation and tympany were recorded. The quality of anesthetic induction for each drug combination was evaluated using previously reported scoring 1-5 scale (Mama et al. 1998). The recovery period was divided into four intervals (table 1). The quality of the recovery was evaluated using modified score mentioned by Bettschart - Wolfensberger et al. (2005).

2.4. Blood tests

Venous blood (8–10 ml) was collected in sterile syringes at zero time (pre-injection) and 45 minutes, 24 and 48 h after injection of the drugs. About 3 ml blood was transferred in potassium ethylene diamine tetra acetic acid (EDTA) containing tubes (2 mg/ml) as an anticoagulant for plasma collection and hematological studies including total leukocyte count (TLC), differential leukocyte count (DLC) and erythrocyte sedimentation rate (ESR). The ESR was determined using the Westergren tube method (Weiss and Wardrop 2010). TLC was determined using a hemocytometer. Blood smears were stained with Giemsa's stain for

differential leukocyte counts. For separation of serum, the rest of blood samples were placed in plain centrifuge tubes. Serum was separated by centrifugation at 3000 RPM for 10 minutes and stored in polypropylene microtubes at -20°C until analysis. Serum was utilized for the measurement of total proteins (TP) and haptoglobin (Hp) (Stockham and Scott 2002). Fibrinogen (Fib) was estimated according to Zargham et al. (1997). Serum concentrations of Hp were determined by ELISA kit (Kamiya, Seattle, USA) using Microplate reader (ChroMate, Anthos Awareness Technology, Palm City, USA). Cellulose acetate electrophoresis was used to quantify serum albumin, alpha, beta and gamma globulins (Rocco 2005).

2.5 Statistical analysis

The data are presented as the means \pm SD. Statistical analysis was performed by analysis of variance (ANOVA) with post hoc testing by Tukey test (Sigma Stat 3.1 for Windows).

RESULTS

3.1. Evaluation of Anaesthesia:

Quality of induction was smooth, rapid, and traumatic in propofol and scored as excellent, whereas in thiopental and ketamine the score was lower but satisfactory (Table 2). Calves receiving propofol showed no excitement or limb movement. Subsequent body movements (head, neck, ear, tail, etc.) were rare. Thiopental showed prolonged period of incoordination, increased muscular activity prior to and during the transition from standing to recumbency. The anaesthesia was characterized by intermittent nystagmus, rapid palpebral and corneal reflexes, ear movement and some muscle twitching at various times.

Induction time was 20 - 50 seconds. Propofol showed short induction time compared to calves receiving thiopental or ketamine. No response to stimulation was recorded in case of propofol anaesthesia from 7 to 22 minute with excellent muscle relaxation. Ocular reflex was abolished and the globe

centralized after 4 minutes and maintained to 19 minutes. In ketamine anaesthesia, calves become usually lateral recumbent within 1 minute and showed no response to stimulation at 8-10 minutes. After induction muscle relaxation was recorded at 15- 19 minutes. Calves administered thiopental were non responsive to stimulation from 7 minutes until 38 minutes of recumbency.

Recovery quality was good in calves receiving ketamine and propofol, while thiopental showed poor recovery. The recovery was without struggling or excitement in propofol. The calves were calm and raised to stand unassisted. All calves were stood on the first attempt and they usually walked normally with little or no ataxia at 30-60 second after standing. In ketamine recovery was uneventful in all animals except 2 calves in which fair recovery was recorded. The calves were standing with some incoordination, which disappeared after 2 minutes. In thiopental calves made vigorous movements of head and neck, and paddled with the legs during sternal

recumbency. Several attempts to stand were recorded. Recovery times were shorter in propofol recipients compared with the other two combinations.

Regurgitation and tympany were not observed when propofol was used. Mild regurgitation was observed 5 minute after induction with ketamine and at 18 minutes in one calf, while in thiopental, it was observed after one, 4 and 12 minutes from achieving lateral recumbency. Mild tympany was occurred in most of calves at the time of recovery in ketamine and thiopental anaesthesia. Hyper salivation was recorded in all calves 7-10 minutes after induction of anaesthesia with propofol and thiopental, whereas in ketamine it occurred after 10 minutes. No apnea was recorded in all combinations.

A significant increase in the heart rate (Fig. 1) was recorded between 5 and 30 minutes in thiopental. However, ketamine and propofol showed no significant changes. The respiratory rate was significantly decreased between 5 and 45 minutes during anesthesia with the three combinations (Fig. 2).

However, the decrease in the respiratory rate was pronounced in thiopental anaesthesia.

3.2. Changes in Leukocytes and ESR:

The mean TLC, absolute numbers of neutrophils, lymphocytes and monocytes and the ESR values for buffalo calves in ketamine thiopental or propofol anaesthesia are presented in Table 3. There was a significant decrease in the total leukocyte and lymphocytes counts in buffalo calves after 45 minutes of ketamine and thiopental anaesthesia compared to the pre-injection values. On the other hand, no significant changes were found in propofol anaesthesia. The absolute number of neutrophils and monocytes did not change significantly in any condition. The ESR showed a significant increase 45 minutes of administration of the three combinations compared to the pre-treatment values.

3.3. Acute phase proteins

The mean values measured for different serum proteins are shown in Table 4. Total proteins and albumin values were significantly low after 45 minutes in all combinations. No significant difference of means was recorded between values of alpha, beta, and gamma globulins in the three combinations.

Pre-treatment plasma Fib and Hp concentrations were low (Table 4). At 45 minutes after injection of anaesthetic drugs, a significant increase ($P < 0.05$) in Fib levels was detected in the all calves compared with pre-injection values (Table 4). Hp levels were significantly high in ketamine combination after 45 minutes, 24 and 48 hours of administration. On the other hand, administration of thiopental and propofol increased Hp levels compared with pretreatment values. This effect was only maintained for the 24-h period after administration. At 48 h after anaesthesia, calves had normal Hp levels.

Discussion

The choice of anesthetic drugs and timing of surgical interventions is important in normal and diseased animals. In the present study, the anaesthetic quality of ketamine, thiopental, and propofol was evaluated in buffalo calves. Xylazine and butorphanol were added to anaesthetic protocol to improve the induction characteristics and the recovery quality.

Propofol at 3 mg/kg provided a smooth, excellent and uneventful induction of anaesthesia combined with good recovery conditions. Similar result was recorded by Frias et al. (2003). Induction of anaesthesia with ketamine was also satisfactory with good muscle relaxation, although induction time was slightly prolonged. This result agrees with that recorded by Pawde et al. (2000). However, the induction quality with thiopental was rated as fair. Thiopental anaesthesia was accompanied by excitement and increased muscle activity as previously recorded (Murison 2001).

Recovery times and duration of anaesthesia were significantly shorter in propofol recipient compared with the other 2 combinations. Similar results were also achieved with propofol in ponies (Nolan et al. 1996). The recovery behaviour was good, no struggling or excitement was seen and most times the animals were calm and coordinate in their unassisted rise to standing posture.

Regurgitation did not occur when calves were anaesthetized with propofol in the present study. However, it occurred in both the thiopental and the ketamine combinations. Hall et al. (2001) suggested that the regurgitation occurred with thiopental and ketamine was due to increased tonic-colonic muscle activity and relaxed cardia. Rapid recovery after propofol injection is desirable in ruminants because extended recumbency enhances the risk of tympany, hypoxemia, regurgitation and aspiration of regurgitated rumen content (Prassions et al. 2005).

Bradycardia was recorded in calves received thiopental. However, ketamine and propofol showed no significant changes in the heart rate. Bradycardia following the administration of thiopental might be caused by inhibition of sympathetic tone from the CNS (Singh et al. 2009). A significant decrease was recorded in the respiratory rate during anesthesia with the three combinations. However, the decrease in the respiratory rate was pronounced in thiopental anaesthesia. These results agree with Mathews et al. (1999) who stated that the respiratory depression was likely the result of a combination of depression of the respiratory center produced by the drug and the effect of recumbency.

All anaesthetic agents have the capacity to influence the functions involved in the acute phase and inflammatory responses; the extent depends on the concentrations, the methodological techniques and the study design used (Schneemilch and Schilling 2004). The acute phase response is a very fast response,

developing before stimulation of the specific immune response and in many cases before the onset of clinical signs. Therefore, it can be considered as one of the earliest markers for any pathologic process or disease. It is highly nonspecific because it develops secondary to numerous conditions (Ebersole and Cappelli 2000). The acute phase response is usually associated with multiple responses, including leukocytes, ESR and APPs (Galley et al. 2000).

In the present study, no significant changes were observed in the total and differential leukocytes counts after propofol anaesthesia. On the other hand, significant decrease was recorded in buffalo calves after 45 minutes and 24 hours of ketamine and thiopental anaesthesia compared to the pre-injection values. Bovine leukocytes contain a greater proportion of lymphocytes and the usual response of cattle to infection or stress conditions is an initial fall in the number of lymphocytes (Latimer et al. 2003). Ketamine was recorded to depress

lymphocyte proliferation and leukocyte count (Cullen and VanBelle 1975). Glucocorticoids released during stressful events are known to cause similar changes in the leukogram (Weber et al. 2004). ESR is a non-specific parameter indicating the presence of an abnormal process in the body and is usually interpreted in a similar way to an abnormal Fib (Jain 1986). The ESR showed significant increase after 45 minutes from injection of the three combinations compared to the pre-injection values.

APPs are widely used as non-specific markers of inflammation (Gabay and Kushner 1999). Numerous articles have been published on the diagnostic and prognostic use of APPs in bovine (Skinner et al. 1991; Horadagoda, et al. 1999; Heegaard et al. 2000). In addition to inflammatory conditions APPs are also released in other conditions such as stress (Lomborg et al. 2008). Various researchers have studied the pro-inflammatory cytokines during anaesthesia (Gilliland et al. 1997; Helmy and Al-Attiyah, 2001; Takaono

et al. 2002). The pro-inflammatory cytokines are difficult to measure and are in the circulation for only a short space of time; therefore it is more appropriate to monitor the APPs as biomarkers for immunological stress (Johnson 1997). Few studies reported the concentrations of APPs during anaesthesia such as ketoprofen (Earley and Crowe, 2002). In this study, blood concentrations of APPs were measured after 45 minutes, 24 and 48 hours of ketamine, thiopental and propofol anaesthesia in buffalo calves.

Fib and Hp are considered as positive APPs in calves (Godeau et al. 2000). In the present work, the Fib concentration in the plasma has been reported to increase significantly in calves only after 45 minutes from induction of anaesthesia by the three combinations. Hp as a sensitive indicator of acute phase response effectively identified the diseased calves more easily because its values in healthy calves were very low compared to those recorded during the inflammatory process (Humblet et al.

2004). In the present experiment, Hp values were significantly increased after 45 minutes, 24 and 48 hours in ketamine recipient calves compared to the pre-injection values. On the other hand, after thiopental and propofol anaesthesia Hp values showed significant increase after 45 minutes and 24 hours, and then decreased back to the normal values after 48 hours. These results indicate that both thiopental and propofol have anti-inflammatory effect. The anti-inflammatory effect of propofol was recorded during endotoxemia (Taniguchi et al. 2000). Crozier et al. (1994) found that IL-6 production after abdominal hysterectomy was both suppressed and delayed in patients receiving total IV anesthesia with propofol. IL-6 is usually classified as a pro-inflammatory cytokine. It is produced by monocytes and macrophages and has important actions on hepatocytes (promoting APPs synthesis) and B lymphocytes, and it may also play a part in wound healing (Cruickshank et al. 1990). Thiopental is also known to have a strong anti-

inflammatory effect through inhibitory action on pro-inflammatory cytokine and nitric oxide. Nitric oxide is an important biological mediator of the inflammatory response, which is produced in a variety of cells as a reaction to receptor stimulation by Nitric oxide synthetase (Galley et al. 1995).

Serum albumin is considered one of the negative acute phase proteins. Decreased concentration was found during acute phase response (Ceron et al. 2005). The comparison of different serum proteins in calves after 45 minutes of anaesthesia with ketamine, thiopental and propofol demonstrated that there was significant decrease in total proteins and albumin values. However, alpha, beta, and gamma globulins showed no significant changes. Although Hp is α 2 globulin, it did not cause significant increase in alpha globulin as Hp is present in normal bovine sera at low levels of 100 μ g/ ml or less and it increased moderately due to anaesthesia (Godson et al. 1996).

These results thus suggest that the APPs may be used to indicate the effect of anaesthetic drugs on pro-inflammatory and acute phase responses in buffalo calves. Hp assay (as a major APP in calves) provided valuable additional information to Fib and hematological investigations. Although ketamine thiopental and propofol anaesthesia has transient and moderate effects in the healthy calves, the choice of anesthetic drugs is more important in immune compromised animals.

In conclusion, IV anesthetic maintenance with propofol in buffalo calves showed better induction and recovery qualities than thiopental and ketamine but the duration of anaesthesia was short. Propofol and thiopental seems to promote anti-inflammatory mechanism indicated by significantly decreased APPs, whereas the addition of ketamine showed significant increase. The ability to alter the inflammatory mechanisms through the selection of a particular anesthetic

technique may have clinical implications in high-risk animals.

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