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Full Length Research Paper

Changes in the haematological profile of the West African hinge-backed Tortoise (Kinixys erosa) anaesthetized with ether or thiopentone sodium

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The effect of ether or thiopentone sodium on haematological parameters of tortoise was determined by evaluating the Packed Cell Volume (PCV), Red Blood Cell (RBC) count, Haemoglobin (Hb) concentration, Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and White Blood Cell (WBC) count of ether or thiopentone-anaesthetized West African Hinge-Backed Tortoise (Kinixys erosa). The blood clotting and sleeping time were also determined. Fifteen tortoises were randomly but equally divided into three groups. Tortoises in Group I were administered with 0.9% Physiological saline, while tortoises in Group II and III were administered with ether and thiopentone sodium respectively. Ether was administered by inhalation while thiopentone sodium was administered intramuscularly. The sleeping time was significantly longer for ether than for thiopentone sodium. The difference in the sleeping time is ascribed to the differences in the physicochemical, pharmacokinetic or pharmacodynamic properties of the two anaesthetics. Blood clotting was delayed in tortoises anaesthetized with ether compared with thiopentone-anaesthetized tortoises, which makes thiopentone a more reliable anaesthetic agent for invasive surgical procedures in the tortoise than ether. The two anaesthetics elicited depression of the haematological parameters of the tortoises with significant ($P<0.05$) decreases in the PCV and RBC values. Hb concentration and MCV were significantly decreased for ether-anaesthetized tortoises, while MCH was significantly decreased for thiopentone-anaesthetized tortoises. The WBC count was elevated in ether-administered tortoises while the value decreased in thiopentone-administered tortoises. The elevation of WBC count was attributed to the irritant effect of ether. It was concluded that ether or thiopentone caused depression of haematological parameters in West African Hinge-Backed tortoise which should be taken into consideration when interpreting values of blood parameters obtained from anaesthetized subjects.

Key words: Ether, thiopentone sodium, haematology, sleeping time, West African hinge-backed tortoise.

INTRODUCTION

The characteristic shell sets the tortoise distinctly apart as a separate order that can not be confused with other animals. They are probably the only reptiles that most humans view with prejudice and the number of people who like the tortoise is surprisingly large. Both terrestrial tortoise and aquatic turtles are often kept as pets in gardens, terrarium and aquarium. As pets, these animals may periodically require evasive or invasive handling for routine Veterinary checks, administration of drugs, exami-nation of the oral or anal orifice, endoscopy, repair and dressing of wounds and fractures, minor and major surgeries, amongst others (Balcombe et al., 2004). Anaesthetic drugs (local or general) may be required to achieve these purposes, several of which have been employed in the tortoise. These include methohexital sodium (Gaztelu et al., 1991; Jackson et al., 2000), atipamezole (Sleeman and Gaynor, 2000; Dennis and Heard, 2002), medetomidine (Sleeman and Gaynor, 2000; Dennis and Heard, 2002), isoflurane and sevoflurane (Heard, 2001),

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ketamine (Holz and Holz, 1994; Greer et al., 2001) and propofol (Heard, 2001; Bertelsen, 2007).

Ether, an inhalant anaesthetic, is known to be fast-acting and cheap, causing minimal physiological effects, but irritating to the respiratory tract (Brunson, 1997) and stressful during the induction period (Van Herck et al., 2001). Thiopentone sodium is an intravenous, short-acting barbiturate, with a rapid onset. It is known to reduce intracranial pressure more effectively than pentobarbital, which is a desirable effect in comatose animals (Cole et al., 2001; Pérez-Bárcena et al., 2005). Reports on the effects of ether or thiopentone on haematological parameters of tortoise is very scarce. The need for this is quite pressing when viewed against the background that sample collection procedures and anaesthesia have been shown to exhibit profound effect on biochemical and haematological effects in animals (Frolich et al., 1996; Heard and Huft, 1998; Dressen et al., 1999), which should be considered when interpreting blood values obtained from anaesthetized subjects. This study was therefore aimed at evaluating haematological changes accompanying anaesthesia in West African Hinge-Backed tortoise (Kinixys erosa) using ether and sodium thiopentone as anaesthetic agents.

MATERIALS AND METHODS

Experimental animals

Fifteen tortoises were purchased and kept at the animal house of Department of Veterinary Physiology and Pharmacology, University of Ibadan. They were fed ripe pawpaw, banana, green vegetables and cooked potato, and had access to clean drinking water *ad libitum*, with normal light: dark timing unaltered. A shallow dish about 10 cm deep was placed in the house to allow the animals to submerge their heads into the water.

Measurement of weight

The body mass of the tortoises was measured to the nearest 5 g with 2 kg Soehnle spring balance.

Induction of anaesthesia and evaluation of sleeping time

Anaesthesia was induced using 20mls of ether by placing the tortoises in anaesthetic chamber containing ether-soaked cotton wool. The time taken from placing of the tortoise in the chamber to when the tortoise began to show first signs of drowsiness was taken as induction time. The sleeping time was taken from the first sign of drowsiness to first sign of recovery from anaesthesia, such as movement of the head or limbs. The tortoises designated for the intravenous anaesthetic agent were weighed and 3 mg/kg of thiopentone was administered via the intramuscular route using the Quadriceps muscle as reported by Bouts and Gasthuys (2002). The sleeping time was recorded as done for the inhalant group.

Blood sample collection and evaluation of blood clotting time

Blood samples were collected by sub-carapacial venipuncture from the tortoises in the three groups following evidence of induction of anaesthesia. A large drop of blood from anaesthetized tortoises was placed on a clean glass slide and mixed continuously with a pin. The time of appearance of the first strand of fibrin was recorded as the blood clotting time. 4 mls of blood was also collected into Lithium-heparinized bottles for haematological analysis. The Packed cell volume (PCV), red blood cell (RBC) count, haemoglobin (Hb) concentration and white blood cell (WBC) count were determined by Cole's method (1986), while the mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were calculated.

Statistical analysis

Student t-test was used to analyze the data (Steel and Torrie, 1996). The differences of the means were considered significant at $P < 0.05$.

RESULTS

Sleeping and blood clotting time

The mean sleeping time in the tortoises anaesthetized with ether was 17.03 ± 2.06 min with average blood clotting time of 4.13 ± 0.15 min. Those anaesthetized with sodium thiopentone had mean sleeping time of 4.30 ± 0.05 min and blood clotting time of 3.05 ± 0.21 min. This was a significant ($p < 0.05$) difference between the mean sleeping times for both groups. Tortoises in the control group had a mean blood clotting time of 3.05 ± 0.06 min, and this was significantly ($p < 0.05$) lesser than that observed for tortoises anaesthetized with ether (Table 1).

Haematological parameters

Red blood cell indices

There was a significant reduction in the mean PCV values of tortoises anaesthetized with ether (18.00 ± 3.54%) and sodium thiopentone (16.13 ± 1.04%) respectively compared with the unanaesthetized tortoises (26.54 ± 0.98%). No significant difference was observed between the mean RBC of unanaesthetized tortoises (0.46 ± 0.02X106/µl) compared with those anaesthetized with ether (0.44 ± 0.03X106/µl), but there was a significant decrease in those anaesthetized with sodium thiopentone (0.37 ± 0.02X106/µl) (Table 2).

The mean Hb of tortoises anaesthetized with ether (8.25±0.44 g/dl) were significantly lower than the unanaesthetized tortoises (11.05±0.51 g/dl), while there was no significant difference between the unanaesthetized tortoises and those anaesthetized with sodium thiopentone (8.95 ± 1.19 g/dl) (Table 2). The mean MCH of tortoises anaesthetized with ether (192.35 ± 22.73 pg) was significantly lower than that of the unanaesthetized tortoises (246.10 ± 11.83 pg), while there was no significant difference compared with the mean MCH of

Table 1. Sleeping and blood clotting time for tortoises anaesthetized with ether or sodium thiopentone.

	Ether-anaesthetized tortoise (n=5)	Thiopentone anaesthetized tortoise (n=5)	Unanaesthetized tortoise (n=5)
Weight (Kg)	0.49±0.26	0.49±0.04	0.49±0.16
Sleeping time (min)	17.03±2.06 a	4.30±0.05a	NA
Clotting time (min)	4.13±0.15a	3.05±0.21	3.05±0.06a

Same superscripts on the same row are significantly (P < 0.05) different; NA - Not applicable.

Table 2. Haematological parameters determined anaesthetized and unanaesthetized tortoises.

Blood parameter	Unanaesthetized group (n=5)	Ether group (n=5)	Thiopentone group (n=5)
PCV %	26.54±0.98ab	18.00±3.54a	16.13±1.04b
RBC (X106/µl)	0.46±0.02a	0.44±0.03	0.37±0.02a
Hb (g/dl)	11.05±0.51a	8.25±0.44a	8.95±1.19
MCH (pg)	246.10±11.83a	192.35±22.73a	238.87±22.68
MCHC (g/dl)	41.68±1.32	52.18±11.82	56.93±9.80
MCV (fl)	596.0±33.27a	422.73±104.69	437.53±28.50a
WBC (X103/µl)	5500±204.39ab	6550±131.39ac	5225±194.05bc

Superscripts on the same row are significantly (P < 0.05) different from each other.

sodium thiopentone (238.87 ± 22.68 pg) (Table 2). The mean MCHC of the unanaesthetized and anaesthetized tortoises was also not significantly (p>0.05) different (Table 2). The MCV of unanaesthetized tortoises (596.0 ± 33.27fl) was significantly higher than that of tortoises anaesthetized with sodium thiopentone (437.53 ± 28.50 fl), but there was no significant difference compared with those anaesthetized with ether (422.73 ± 104.69 fl) (Table 2).

White blood cell count (WBC)

The mean WBC of unanaesthetized tortoises (5500 ± 204.39X103/µl) was significantly lower than that of those anaesthetized with ether (6550 ± 131.39X103/µl) and significantly higher than that observed for those anaesthetized with sodium thiopentone (5225 ± 194.05X103/µl). There was a significant difference between the tortoises anaesthetized with ether and sodium thiopentone (Table 2).

DISCUSSION

In this study, the sleeping time was significantly longer for ether, which is an inhalant anaesthetic agent, than for thiopentone sodium, an intravenous anaesthetic. The difference in the sleeping time is unarguably ascribable to differences in the physicochemical, pharmacokinetic or pharmacodynamic properties of ether and thiopentone. Certain considerations needed to be taken when choosing anaesthetic as chemical restraint in reptiles. Trkova et al. (2008) submitted that the induction and recovery periods should be as short as possible and Heard (2001) recommended inhalation anaesthesia as the technique of choice with respect to minimizing side effects. These considerations place ether as drug of choice as oppose to thiopentone. However, Girling and Raiti (2004) strongly recommended injectable rather than inhalant anaesthetic on the ground that inhalation anaesthesia being administered by either mask or tracheal intubation, or a combination of both often leads to animals struggling or difficulties with restraint. The only reservation raised by Schumacher and Yelen (2006) about injectable anaesthetic is the prolonged recovery phase, which is really not applicable to thiopentone sodium being an ultra short barbiturate (Hung et al., 1992); a fact further confirmed in West African Hinge-Backed Tortoise in this study.

Blood clotting was delayed in tortoises anaesthetized with ether-compared with thiopentone-anaesthetized tortoises. The mean body weight of the tortoises used in this study though lower than what was reported by Oyewale et al. (1998) was consistent for the three groups, therefore the difference in the blood clotting time may not be due to such factors like weight or age as reported by Chaloupka and Musick (1996) or Bradley et al. (1998) but to varying individual effects of the two anaesthetics used. Dordoni et al. (2004) had actually reported that thiopentone sodium reduced platelet function *in vivo* and *in vitro*, consequently prolonging blood clotting time. The findings in this study however showed that ether prolonged blood clothing process much more than thiopentone in tortoises. This therefore makes thiopentone a much more reliable anaesthetic agent for invasive surgical procedures in the tortoise because of its faster blood clotting time, which is an important consideration in such type of surgical interventions (Furie and Furie, 2005).

The two anaesthetics used in this study generally elicited depression of the haematological parameters of the tortoises with significant decreases in the pack cell volume in the test animals. The depression of RBC values was not significant but haemoglobin concentration and mean corpuscular volume were significantly decreased for ether-anaesthetized tortoises, while mean corpuscular haemoglobin was significantly decreased for thiopentone-anaesthetized tortoises.

Anaesthetics-induced depression of the haematological parameters has similarly been reported in other reptiles like Iguana (Knotkova et al., 2006) or in mammals such as rats (Deckardt et al., 2007), boar (Golemanov et al., 1986) or sheep (Edjtehadi, 1978). These changes occur within 15 min and begin to return to normal by 45 min after induction (Dressen et al., 1999); and are caused by anaesthetic-induced splenic vasodilatation resulting in sequestration of blood cells (Marini et al., 1994).

In this study, the erythrocytes were found to be microcytic, a condition thought to be responsible for relative elevation of the values of the mean corpuscular haemoglobin concentration of the tortoises. Every of the parameter responded in the same direction for the ether- or thiopentone-administered tortoises except the white blood cell count which was elevated in ether-administered tortoises while it decreased in thiopentone-administered tortoises. It is difficult to establish the reason for this difference which was also demonstrated by Golemanov et al. (1986) with thiopentone increasing white blood cell count in boar and on the other hand causing a decrease in sheep (Edjtehadi, 1978). It is generally admissible that anaesthetics lower RBC, WBC, PCV, Hb concentration due to splenic and capillary sequestration (Niezgoda et al, 1987; Heard and Huft, 1998; Apple et al., 1993; Knotkova et al., 2006). It has also been shown specifically that anaesthetics exhibit anti-inflammatory effects (Kenyon et al., 1985; O' Donnel et al., 1992; Singh, 2003), but the added component of stress attendant to the strenuous process of administration of anaesthetics especially the inhalant agent, serve to counteract the lowering of white cell count in anaesthetized subjects (Wall, 1985). More specific is the fact that the irritant effect of ether on the respiratory tract (Brunson, 1997) and its stressful effect during induction period (Van Herck et al., 2001) are capable of elevating white blood cell count during anaesthesia as observed in this study.

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