

EFFECT OF ISOFLURANE AND HALOTHANE ON MYOCARDIAL FUNCTION IN HEALTHY DROMEDARY CAMELS AS ASSESSED BY CARDIAC TROPONIN I

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ABSTRACT

The effect of general anaesthesia with isoflurane and halothane on serum concentration of cardiac troponin I (cTnI) in healthy dromedary camels was determined. Six healthy female camels were premedicated with xylazine and anaesthesia was induced with ketamine and maintained with either isoflurane (isoflurane group, $n=6$) or halothane (halothane group, $n=6$). A washout period of 2 weeks was allowed between the two anaesthetic protocols. Nine blood samples (T0-T8) were obtained from each camel in heparinised tubes to determine arterial and venous blood gases and pH, and in plain tubes to obtain serum for cTnI analysis. Blood samples were collected immediately before anaesthesia (T0), 20min after xylazine administration (T1), 20min after ketamine administration (T2), 60min of inhalation anaesthesia (T3), 40 and 80min during recovery (T4 and T5) and 24, 48 and 72h after recovery (T6-T8). In isoflurane group, serum cTnI concentrations did not rise above 0.04ng/mL. On the other hand, in halothane group, serum cTnI concentrations increased markedly after 40 and 80min of recovery to be 0.20 and 0.47ng/mL, respectively. Serum cTnI concentrations remained significantly elevated at 24h and 48h after recovery. Comparing halothane group to isoflurane group, mean serum concentration of halothane cTnI was significantly higher at 40 and 80min of recovery and at 24h and 48h after recovery. In conclusion this study proved that halothane has marked effect on cardiomyocytes in healthy camels compared to isoflurane. Therefore, the use of halothane should be restricted in camels with suspected cardiac diseases.

Key words: Anaesthesia, camels, cardiac troponin I, halothane, isoflurane

Cardiac troponin I (cTnI), the “gold standard” for the non-invasive diagnosis of myocardial injury, is a protein found in the myocardial cell that initiates tropomyosin contraction (Archer, 2003; Burgener *et al*, 2006; O’Brien, 2006; Ladenson, 2007; Wells and Sleeper, 2008; Plebani and Zaninotto, 2009; Kraus *et al*, 2010). Its serum concentration elevates after acute myocardial injury because of leakage from the damaged myocardial cells (O’Brien, 2006). The degree of increase in cTnI has been shown to correlate with the extent of myocardial damage and with survival in humans (Stanton *et al*, 2005) and animals (Ricchiuti *et al*, 1998; Oyama and Sisson, 2004; Fonfara *et al*, 2010). In camels, cTnI is elevated in animals during racing (Tharwat *et al*, 2013b) and when being subjected to long road transportation (Tharwat *et al*, 2013a).

General anaesthesia is associated with a relatively high risk of morbidity and mortality in animal species. Cardiovascular compromise is an important contributor to mortality and morbidity (Brodbelt *et al*, 2007). Anaesthesia may result in complex changes in cardiovascular function, such as cardiovascular depression with hypotension and reduced tissue perfusion, and in turn, reduced myocardial oxygen delivery and cellular damage. Anaesthesia, therefore, may represent a potential risk for the myocardium (Cilli *et al*, 2010). Increased cTnI after anaesthesia relative to pre-anaesthesia levels was observed in a number of apparently healthy dogs undergoing anaesthesia (Cilli *et al*, 2010; Verbiest *et al*, 2013). On the other hand, Slack *et al* (2011) reported that uncomplicated general anaesthesia in horses, with or without surgery, does

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not affect the concentration of cTnI in the first 24h postoperatively.

Myocardial injury secondary to ischaemia is not limited to patients with coronary artery disease, but can occur at any time when coronary blood flow is inadequate to meet myocardial oxygen demands. Both inhalational and intravenous anaesthetics can affect coronary blood flow and, therefore, have the potential to initiate cTnI release from the myocardial cell (Ramanathan and Skinner, 2005). Since many critically ill camels undergo general anaesthesia for a variety of reasons, it is important to determine the effects, if any, general anaesthesia has on serum cTnI concentrations. Hence, the aim of the present study was to determine the effect of halothane or isoflurane general anaesthesia after xylazine and ketamine administration on the myocardial function in healthy camels as assessed by cTnI.

Materials and Methods

Camels

The experimental protocol was approved by the Ethics Committee for Animal Research of the Scientific Research Deanship of Qassim University in Saudi Arabia. The design of the study has been partially reported in a parallel study (Al-Sobayil *et al*, 2013). Six adult female dromedary camels (*Camelus dromedarius*) were used. The animals were considered healthy on the basis of physical examination (auscultation of the heart, lungs, rumen and intestine, and measurement of heart rate, respiratory rate and rectal temperature), laboratory evaluation (normal complete blood cell counts and biochemistry panel), echocardiography, and electrocardiography. Their mean body weight was 378 kg (range = 321-503 kg) and their mean age was seven years (range = 5-12 y). The experiment was divided into two parts, each using this group of six camels. Food and water were withheld 48 h and 24 h, respectively, before each trial. The experiments were performed in a temperature-controlled room maintained at 21-24°C.

Anaesthesia

Sixteen-gauge intravenous and 20-gauge intra-arterial catheters (Mais Co., Riyadh, Saudi Arabia) were placed in the left jugular vein and the auricular artery (occasionally the radial artery), respectively, after clipping, surgical scrubbing and local infiltration of the skin with 1 ml 2% lidocaine (Norbrook Laboratories, UK).

The camels were premedicated with xylazine HCl (0.2 mg/kg, IV, Bomazine 10%, BOMAC

Laboratories Ltd., New Zealand). Twenty minutes later, anaesthesia was induced with Ketamine (2 mg/kg, IV, Ketamine 10%, Alfasan, Woerden, Holland) and was maintained with either isoflurane (Floran, HIKMA Pharmaceuticals, Amman, Jordan) or halothane (Anestane®, HIKMA Pharmaceuticals, Amman, Jordan) 100% oxygen at a flow rate of 6 L/min. The animals were first subjected to isoflurane and then to halothane anaesthesia, with a washout period of 2 weeks between the two anaesthetic protocols.

The anaesthetised camel was moved onto a padded operating table, positioned in right lateral recumbency and was connected via the endotracheal tube with a semiclosed-circle rebreathing anaesthetic machine (SurgiVet Foal Circuit Set, Smith Medical North America, Waukesha, WI, USA). Anaesthesia was discontinued after 1 h and the camels received supplemental oxygen (6 L/min) through the endotracheal tube. After tracheal extubation, oxygen was insufflated through a nasal tube until sternal recumbency was achieved. Respiratory and heart rates and arterial blood pressure were measured at the nine time points (T0-T8) of blood sampling as described below. Blood pressure was indirectly measured with an oscillometric technique (Accutorr Plus™ Recorder, Datascope, Datascope Corp., Paramus, NJ 07652, USA) using a cuff placed around the tail.

Blood sampling and blood gas analysis

Throughout this study, nine blood samples in plain tubes (T0-T8) were obtained from each camel for serum cTnI analysis. The first blood sample was collected immediately before anaesthesia (T0), the second (T1) was collected 20 min after the xylazine administration (premedication) and the third blood sample (T2) was collected 20 min after the ketamine administration (induction). The fourth blood sample (T3) was collected after 60 min of inhalation anaesthesia. Two blood samples (T4 and T5) were collected during 40 and 80 min of recovery, respectively. Blood samples T6-T8 were collected 24, 48 and 72 h after recovery from anaesthesia. Parallel, arterial blood samples were collected in heparinised tubes for the immediate measurements of arterial pH, PO₂ and PCO₂ using a blood gas analyser (GEM® Premier 3000, Instrumentation Laboratory Co., Bedford, MA, USA).

Cardiac troponin I assay

Cardiac troponin I was analysed in serum as was recently reported (Tharwat *et al*, 2013a,b) using

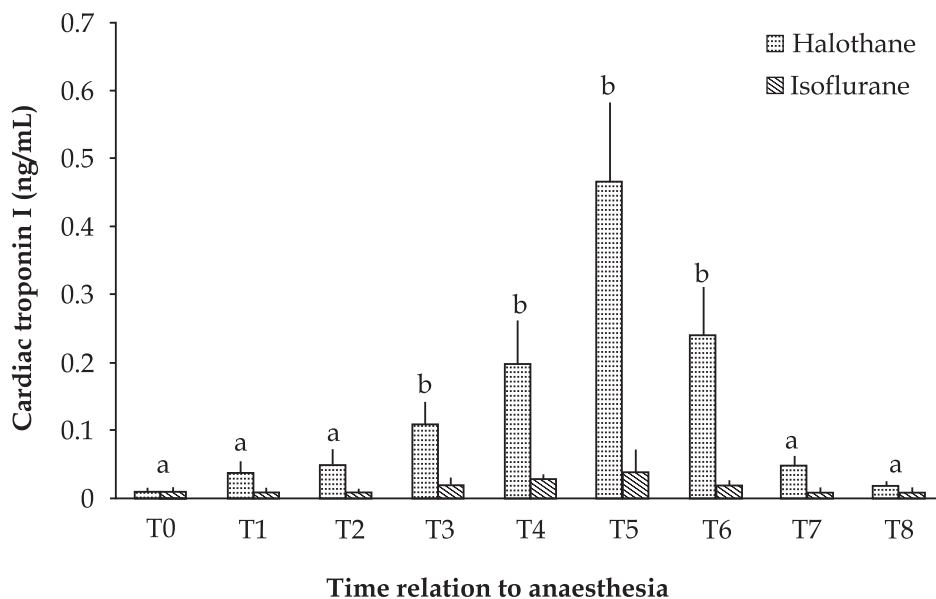


Fig 1. Pre-anaesthetic, anaesthetic and post-anaesthetic serum concentration of cardiac troponin I (means \pm SEM) in camels ($n = 6$) undergoing isoflurane and halothane anaesthesia. T0, immediately before anaesthesia; T1, 20 min after xylazine administration; T2, 20 min after ketamine administration; T3, 60 min during inhalation anaesthesia; T4, 40 min of recovery; T5, 80 min of recovery; T6-T8, 24 h, 48 h and 72 h after anaesthesia. b Differs significantly between the two anaesthetic agents at $P < 0.05$.

a point-of-care analyser (VetScan i-STAT® 1, Abaxis, California, USA) according to the manufacturer's instructions. This analyser employs a two-site enzyme-linked immunosorbant assay (ELISA). All results are expressed as nanograms per milliliter (ng/mL) with an intra-assay coefficient of variance of 5%. The lower limit of detection of cTnI for this assay was 0.02 ng/mL. The i-STAT cTnI test reports 0.00 to 50.00 ng/mL. Samples above the reportable range will yield ">50.00 ng/mL" on the analyser display screen. However, the performance characteristics of the i-STAT cTnI measurement have not been established for cTnI values above 35.00 ng/mL. Values < 0.02 ng/mL cannot be discriminated, although the analyser provides a specific point estimate of 0.00, 0.01 or 0.02 ng/mL.

Statistical method

Data are presented as means \pm SEM, and were analysed statistically using the SPSS statistical package (SPSS, Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, USA, Copyright© for Windows, version 18.0, 2009). A repeated measures analysis of variance was employed as the statistical model to evaluate the differences over time in the isoflurane and halothane anaesthetised camels. The Duncan test was used to calculate multiple comparisons. Student t test was used to evaluate

differences between isoflurane and halothane anaesthetised animals. Results were considered significant at $P < 0.05$.

Results

In the halothane-anaesthetised camels, the heart rate decreased insignificantly after xylazine (T1) and highly significantly ($P = 0.0001$) after ketamine (T2) administration; it returned to baseline value during inhalation anaesthesia. In the isoflurane-anaesthetised camels, the heart rate decreased significantly ($P = 0.035$) 80 min into recovery (T5). The respiratory rate decreased significantly ($P = 0.036$) in the isoflurane group after xylazine administration (T1), and in the halothane group at T4, 40 min into recovery ($P = 0.009$). The mean blood pressure decreased significantly ($P = 0.0001$) during halothane and isoflurane anaesthesia at T3 and returned close to the baseline value during recovery in the halothane group; however, it increased significantly after 80 min of recovery (T5) in the isoflurane-anaesthetised camels.

In the halothane group, there was a significant ($P = 0.0001$) decrease in venous pH at T2 and T3 and in arterial pH at T3. Additionally, there was a significant ($P = 0.014$) decrease in venous pH at T4. These returned to baseline value at T5. Although there were insignificant changes in arterial PCO_2 ,

venous PCO₂, venous PO₂ and arterial PO₂ showed a significant (P = 0.0001) increase during halothane anaesthesia (T3). In addition, there was also a significant (P = 0.045) increase in arterial PO₂ after 40 min during recovery (T4). On the other hand, there were insignificant (P>0.05) changes in blood gases and pH during isoflurane anaesthesia.

Fig 1 summarises the mean serum concentrations of cTnI in the isoflurane and halothane groups throughout the T0-T8 time points. For the isoflurane and halothane groups, the mean serum resting basal cTnI value in the camels before anaesthesia was 0.01 ± 0.01 ng/mL. In the isoflurane group, although the serum concentrations did not increase above 0.04 ng/mL, the increased serum cTnI values were significant at 60 min of inhalation anaesthesia and at 40 and 80 min (T3-T5) of recovery (P = 0.002, 0.0002 and 0.0002, respectively). In the halothane group, however, serum cTnI concentrations increased markedly at 40 and 80 min of recovery (T4 and T5) to reach a value of 0.20 and 0.47 ng/mL, respectively (P = 0.03). The serum cTnI concentration remained significantly elevated at 24 h and 48 h after recovery from anaesthesia (P = 0.04). Comparing the two anaesthesia groups, the mean serum concentrations of halothane cTnI was significantly higher after 40 and 80 min of recovery and at 24 h (P = 0.04) and 48 h (P = 0.03) post-recovery from anaesthesia.

Discussion

To the authors' knowledge, this is the first study to evaluate the effect of general anaesthesia by either isoflurane or halothane on myocardial function in healthy camels by assessment of cTnI serum concentrations at pre-anaesthetic, anaesthetic and post-anaesthetic (0 to 72 h) periods. Before anaesthesia, the mean serum cTnI concentration in both the halothane and isoflurane groups was 0.01 ± 0.01 ng/mL, a similar result to that recently reported by Tharwat *et al* (2013 a,b) in healthy camels (0.01- 0.07 ng/mL). After anaesthesia, cTnI in the isoflurane- anaesthetised group had increased to 0.04ng/mL at 60 min of inhalation anaesthesia and at 40 and 80 min of recovery, a value, although differing significantly from the pre-anaesthetic value, was still within the normal reference range for healthy camels. In contrast, serum concentration of cTnI in the halothane-anaesthetised group had increased significantly beyond the reference range for camels, with 0.20 and 0.47 ng/mL at 40 and 80 min, respectively, of recovery. In addition, the serum values of cTnI remained significantly elevated

for the followings 24 and 48 h after recovery from anaesthesia.

The lack of increase in cTnI concentrations outside the reference range in the isoflurane-anaesthetised camels represents a true absence of myocardial injury and is similar to results previously reported in healthy women (Erol and Ozen, 2007), horses (Slack *et al*, 2011) and dogs (Pelander *et al*, 2008; Saunders *et al*, 2009; Cilli *et al*, 2010) maintained with isoflurane general anaesthesia. In the human study, normal post-anaesthetic cTnI concentrations were found in all women maintained with either isoflurane and N₂O inhalation anaesthesia or propofol and fentanyl intravenous anaesthesia. In the horse study, all of the horses maintained with isoflurane or desflurane general anaesthesia had cTnI values within the post-anaesthetic reference range, and only 21% developed detectable cTnI 6 or 12 h following anaesthesia.

In two of the dog studies (Pelander *et al*, 2008; Saunders *et al*, 2009), 90% of the dogs undergoing either castration or ovariohysterectomy and 87% of the dogs undergoing hysterectomy had cTnI concentrations within the reference range post-anaesthesia. In a third dog study (Cilli *et al*, 2010), 79% had undetectable cTnI concentrations following anaesthesia. For maintenance of anaesthesia, some of the dogs in the above studies received isoflurane or desflurane, while others received sevoflurane or halothane. Some of the dogs in the above-mentioned studies experienced extreme changes in heart rate, hypotension, haemorrhage and hypoxia, and these may have been the reason for the elevated cTnI concentrations in a small proportion of the dogs. Similarly, in the horse study, three animals that were excluded from statistical analysis because of colic, myopathy and severe haemorrhage had cTnI concentrations above the reference range at one or more time points. Concerning the dog studies, it is important to note that cTnI was determined using a different technique, the IMMULITE assay, with a lower detection limit of 0.2 ng/mL. The assay used in the present study was, however, a third-generation cTnI analyser with a lower limit of detection (0.01 ng/mL) and a higher sensitivity and, consequently, resulting in a more accurate detection of cTnI.

In a recent study in dogs undergoing general anaesthesia for orthopedic surgical procedures with isoflurane (Verbiest *et al*, 2013), 55% of the dogs had a post-anaesthetic increase of cTnI concentration compared to their pre-anaesthetic values, whereas, a decrease of cTnI concentration was observed in

11% of the dogs. Some dogs with increased post-anaesthetic cTnI concentrations suffered from brady- or tachycardia and hypo- or hypertension during anaesthesia. These complications might have contributed to the post-anaesthetic increase of cTnI. However, the authors also stated that these complications were observed in dogs that had not experienced an increase of cTnI concentration. In the isoflurane group of this study, bradycardia was found only at time points T1-T2, hypotension at T3 and hypertension at T4 and T5. Hypotension during anaesthesia leads to reduced coronary blood flow, myocardial ischemia and consequently, myocardial cell compromise which could eventually result in the release of cTnI (Verbiest *et al*, 2013).

On the other hand, in the halothane-anaesthetised camels, the serum concentration of cTnI increased significantly beyond the reported reference range for camels, indicating cardiac injury for up to the 48 h post-anaesthesia. The arterial and venous pH decreased significantly at time points T2-T4, and the venous PCO₂ increased steadily post-anaesthetic to maximise at the time point T3. In addition, the heart rate decreased at T1-T2, and the blood pressure decreased significantly at T3. In horses, the effects of halothane and isoflurane anaesthesia on central and peripheral hemodynamics have been extensively studied, and both agents were observed to produce dose-dependent decreases in cardiac function (Steffey and Howland, 1978, 1980). However, at constant arterial carbon dioxide tension (PaCO₂), halothane has been reported to produce a greater depression of cardiac function than isoflurane anaesthesia (Steffey and Howland, 1980). This was thought to result from a greater depression of myocardial contractility by halothane than by isoflurane (Brunson, 1990).

During intermittent positive-pressure ventilation, cardiovascular depression has been cited as being significantly less during isoflurane anaesthesia than during halothane anaesthesia (Steffey and Howland, 1980). In addition, the previously reported superior cardiovascular function during isoflurane compared to halothane anaesthesia has been maintained in horses undergoing surgery (Blissitt *et al*, 2008). The cardiac index was reported to be significantly higher during isoflurane anaesthesia than during halothane anaesthesia. In addition, compared to halothane anaesthesia, during isoflurane anaesthesia, femoral arterial blood flow was reported to be significantly higher in both pelvic limbs (Raisis *et al*, 2005).

Conclusions

Although limited by the small number of animals in the study, the findings show that healthy camels have mildly elevated and significantly elevated cTnI with isoflurane and halothane anaesthesia, respectively; however, in the isoflurane group the upper limit for the camel reference range was not exceeded. The measurement of cTnI would therefore appear to be useful in assessing intraoperative myocardial cell compromise in camels. The cause of the cardiac cell compromise during halothane anaesthesia in this study was likely due to extreme changes in heart rate and blood pressure, and the increased arterial concentration of PCO₂. According to the cardiac biomarker cTnI value, it seems that isoflurane is superior to halothane as an inhalation anaesthetic in dromedary camels especially in those with suspected cardiac diseases.

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