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RESEARCH PAPER

Urinary neutrophil gelatinase-associated lipocalin concentration changes after acute haemorrhage and colloid-mediated reperfusion in anaesthetized dogs

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Abstract

Objective To determine changes in urine neutrophil gelatinase-associated lipocalin concentration (uNGAL) in anaesthetized Greyhound dogs that developed acute tubular damage following haemorrhage and resuscitation with colloid-based fluids.

Study design Prospective experimental study.

Animals Seven healthy adult entire male Greyhound dogs.

Methods During isoflurane anaesthesia, approximately 50 mL kg^{-1} of blood was removed to maintain mean arterial pressure (MAP) ≤40 mmHg for 1 hour followed by gelatin-based colloid administration to maintain MAP ≥60 mmHg for 3 hours. Data, including oxygen extraction ratio and uNGAL, were collected before (T0) and immediately following (T1) haemorrhage, and hourly during reperfusion (T2–T4). After T4, dogs were euthanized and renal tissue was collected for histology. Statistical analysis was performed using repeated-measures one-way ANOVA. Data are presented as means (95% confidence interval).

Results Histology identified renal tubular epithelial damage in all dogs. Urine NGAL concentration increased from 12.1 (0–30.6) ng mL^{-1} at T0 to 122.0 (64.1–180.0) ng mL^{-1} by T3. Compared with T0, uNGAL was significantly higher at T3 ($p = 0.016$) and was increased 24-fold.

Conclusions and clinical relevance Despite wide individual variation in baseline uNGAL, increases in uNGAL were observed in all dogs, suggesting that this biomarker has the potential to detect renal tubular injury following haemorrhage-induced hypotension and colloid-mediated reperfusion.

Keywords acute kidney injury, dogs, hypotension, ischaemia, NGAL.

Introduction

Acute kidney injury (AKI), a common syndrome in human and veterinary medicine, can be caused by ischaemia–reperfusion (IR) injury subsequent to renal hypoperfusion during general anaesthesia (Behrend et al. 1996; Moore et al. 2012; Mugford et al. 2013). Four sequential stages of AKI have been identified: initiation, extension, maintenance and recovery (Devarajan 2006; Basile et al. 2012; Mugford et al. 2013). Animal studies have shown that therapeutic interventions to reverse or prevent further progression of AKI must occur during the initiation or extension phases (Devarajan 2006).

Unfortunately, AKI diagnosis and staging to date have relied upon indicators of glomerular filtration deficiency [e.g. serum creatinine concentration (SCr), urine output] that do not change significantly until the maintenance phase, 2–3 days following initiation of injury (Basile et al. 2012). Late diagnosis and delayed intervention contribute to the high mortality and morbidity associated with AKI (Basile et al. 2012; Moore et al. 2012).

Early diagnosis of AKI might be possible by measuring urinary neutrophil gelatinase-associated lipocalin (NGAL), a biomarker of renal tubular injury. Urine NGAL concentrations (uNGAL) are increased 2 hours after initiation of IR AKI in rodent models using unilateral or bilateral renal occlusion (Mishra et al. 2003; Mori et al. 2005). Clinical studies in people placed on cardiopulmonary bypass demonstrate increases in uNGAL within 2 hours of renal IR (Mishra et al. 2005; Bennett et al. 2008). While clinical studies in dogs report increases in uNGAL 12 hours after injury (Lee et al. 2012; Steinbach et al. 2014), whether uNGAL can detect tubular injury in dogs at earlier time points has not been reported.

The aim of this study was to document uNGAL changes in anaesthetized dogs using a model of renal injury produced by haemorrhage and subsequent colloid fluid resuscitation. We hypothesized that the model would produce histological evidence of renal tubular damage and that uNGAL would increase from baseline within 3 hours of renal hypoperfusion.

Materials and methods

Animals and selection criteria

Seven adult entire male Greyhound dogs, median (range) body weight 33 (30–35) kg, donated to the hospital and scheduled to be used as terminal blood donors were included in the study. Dogs were included if physical examination, renal ultrasonography, urinalysis and complete blood count were normal, and SCr, blood urea nitrogen and serum albumin concentrations were within normal reference intervals previously reported for healthy adult Greyhounds (Zaldívar-López et al. 2011). Ethics approval was granted by the Murdoch University Animal Ethics Committee (no. R2586/13) and the dogs were cared for according to the *Australian Code for the Care and Use of Animals for Scientific Purposes*.

Anaesthesia

Dogs were fasted for at least 8 hours prior to the procedure. Water was provided *ad libitum* until premedication. Methadone (0.3 mg kg^{-1}; Ilium Methadone, 10 mg mL^{-1}; Troy Laboratories, NSW, Australia) was administered intramuscularly (IM) 30 minutes prior to induction of general anaesthesia by intravenous (IV) alfaxalone (1.7–2.2 mg kg^{-1}; Alfaxan Injection, 10 mg mL^{-1}; Jurox, NSW, Australia). Following orotracheal intubation, dogs were positioned in left lateral recumbency. Anaesthesia was maintained with isoflurane (ISO; Veterinary Companies of Australia, NSW, Australia) in an oxygen and medical air mix providing an inspired oxygen concentration (FIO_2) of 30% via a circle system. End-tidal isoflurane concentration (F_E′Iso) was maintained at 1.3–1.4% [1 × minimum alveolar concentration (MAC)] (Steffey & Howland 1977; Valverde et al. 2003). Hartmann's solution (Compound Sodium Lactate; Baxter Healthcare, NSW, Australia) 10 mL kg^{-1} $hour^{-1}$ IV and fentanyl (Fentanyl injection, 50 μg mL^{-1}; AstraZeneca, NSW, Australia) 2 μg kg^{-1} $hour^{-1}$ IV were administered throughout anaesthesia. The dogs were mechanically ventilated to maintain arterial carbon dioxide tension ($PaCO_2$) at 35–45 mmHg (4.7–6.0 kPa) (Model TH-1; Beijing Read Eagle Technology Co. Ltd, China). Active warming (Bair Hugger Warming Unit Model 505; Arizant Healthcare Inc., MN, USA) maintained oesophageal temperature between 36.5 and 37.5 °C (Surgivet V9203; Smiths Medical, MA, USA).

Instrumentation

A 12 gauge, 13 cm cannula (Angiocath; Becton Dickinson Infusion Therapy Systems Inc., NJ, USA) was inserted into the cranial vena cava via the right jugular vein for collection of blood samples and injection of lithium chloride. The left femoral artery was cannulated (20 gauge, 3 cm Angiocath; Becton Dickinson Infusion Therapy Systems Inc.) to facilitate measurement of mean arterial pressure (MAP), measurement of lithium chloride voltage for cardiac output ($\dot{Q}t$) calculation and removal of blood to generate experimental haemorrhage. A nerve stimulation-guided (Innervator 272; Fisher & Paykel Health Care, New Zealand) femoral nerve block was performed at the femoral triangle with bupivacaine (0.1 mL kg^{-1}; Bupivacaine hydrochloride, 50 mg