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Characterization of kidney damage using several renal biomarkers in dogs with naturally occurring heatstroke

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ABSTRACT

Heatstroke is often associated with acute kidney injury (AKI). The objectives of this study were to characterize the kidney damage occurring in canine heatstroke using routine and novel biomarkers and to assess their diagnostic and prognostic performance. Thirty dogs with naturally occurring heatstroke were enrolled prospectively. Blood and urine specimens were collected at presentation, at 4 h post-presentation and every 12 h until discharge or death. The glomerular filtration rate (GFR) and electrolyte fractional excretion (FE) at 4 h post-presentation were also calculated, based on urinary clearances. AKI was further characterized by evaluating urine neutrophil gelatinase-associated lipocalin/creatinine ratio (UNGAL), urine retinol-binding protein/creatinine ratio (URBP), urine C-reactive protein/creatinine ratio (UCRP) and urine protein to creatinine ratio (UPC). These biomarkers were compared to those for 13 healthy dogs.

Thirteen dogs (43%) died and 17 (57%) survived. Median serum creatinine concentration at presentation was 1.69 mg/dL (range, 0.5–4.7 mg/dL), while concurrent GFR was markedly decreased (median 0.60 mL/min/kg; range, 0.00–3.10 mL/min/kg). Median Na fractional excretion was 0.08 (range, 0.01–0.41) and was an accurate predictor of AKI (area under curve 0.89; 95% confidence intervals 0.76–1.00). Median UPC at presentation was 4.8 (range, 0.4–46.0). Median UCRP, URBP and UNGAL were increased in all dogs with heatstroke, and were mean 232, 133, and 1213-fold higher than healthy control dogs, respectively. In conclusion, although AKI occurs invariably in dogs with heatstroke, it is often subclinical at presentation. Damage occurs in both the renal tubules and the glomeruli. Novel kidney function tests for the characterization of renal injury and its severity are superior to conventional markers and could be used to facilitate early diagnosis of AKI.

Introduction

Heatstroke in dogs is a severe syndrome, characterized by core body temperatures >41 °C and central nervous system abnormalities. It results from the inability to dissipate accumulated heat either after exposure to a hot humid environment (classical heatstroke) or after strenuous physical exercise (exertional heatstroke; Flournoy et al., 2003; Bruchim et al., 2006; Epstein and Roberts, 2011). Heatstroke manifests as hyperthermia, tachypnea, panting, collapse, shock, altered consciousness, seizures, vomiting, diarrhea, petechiae and ecchymosis (Bruchim et al., 2006) and can result in acute kidney injury (AKI), systemic inflammatory response syndrome (SIRS), disseminated intravascular coagulation, and multiple organ failure (Flournoy et al., 2003; Roberts et al., 2008; Bruchim et al., 2009; Leon and Helwig, 2010; Heled et al., 2013). Mortality rates can exceed 50% (Drobatz and Macintire, 1996; Varghese et al., 2005; Bruchim et al., 2006).

AKI represents a spectrum of conditions associated with the sudden onset of renal parenchymal injury (Langston, 2010). The pathogenesis of heatstroke-associated AKI is most likely multifactorial, and includes decreased perfusion due to dehydration and hypovolemia, direct thermal injury, rhabdomyolysis-associated myoglobinuria and SIRS (Heled et al., 2013). Although AKI can be mild and go unnoticed, overt kidney failure often ensues (Lin et al., 2003; Langston, 2010). International Renal Interest Society (IRIS) grade 1 AKI represents mild injury,[1] with serum creatinine concentration (sCr) still within the reference interval; therefore, diagnosis can be challenging using routine biochemical and functional parameters. Renal biomarkers can potentially promote the early detection of AKI and injury localization (i.e. glomerular vs. tubular; Maddens et al.,

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[1] See: International Renal Interest Society http://www.iris-kidney.com (accessed 29 June 2015).

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2011; Smets et al., 2012; De Loor et al., 2013; Kai et al., 2013; Segev et al., 2013; Steinbach et al., 2014).

Kidney functional markers can be classified into those measured routinely (e.g. sCr), and those that can be calculated using routine biochemistry results, such as glomerular filtration rate (GFR), endogenous creatinine clearance and electrolyte fractional excretion (FE). Novel urinary biomarkers for kidney injury include neutrophil gelatinase-associated lipocalin (NGAL), C-reactive protein (CRP) and retinol-binding protein (RBP). NGAL is a 25 kD protein, originally discovered in neutrophils, that forms a covalent heterodimeric complex with gelatinase (Kjeldsen et al., 1992, 1993). The renal tubular expression of NGAL is usually low, but markedly increases with renal tubular cell injury (Mori and Nakao, 2007). It is one of the earliest, most robustly induced proteins in both humans and animals with AKI (Mishra et al., 2004; Mori and Nakao, 2007; Lee et al., 2012; Nabity et al., 2012). RBP is a low molecular weight protein filtered freely and completely reabsorbed by renal tubules, rendering it a marker of renal tubular function (De Loor et al., 2013). Conversely, CRP is a relatively high molecular weight protein which is normally not filtered through the glomerulus (De Loor et al., 2013). Thus, urinary CRP concentration reflects changes in glomerular capillary permselectivity (Martinez-Subiela et al., 2013).

In this study, we hypothesized that heatstroke would lead to severe AKI due to both glomerular and tubular damage, thereby increasing the concentrations of routine and novel renal biomarkers. Our objectives were to characterize kidney damage in dogs with naturally occurring heatstroke using routine and novel biomarkers and to assess their diagnostic and prognostic utility.

Materials and methods

Animals

The study was approved by the Koret School of Veterinary Medicine Institutional Animal Care and Use Committee (Approval number KSVM/VTH_2011). Dogs presented to the Emergency Service, Hebrew University Veterinary Teaching Hospital (HUVTH) between 2011 and 2013 and diagnosed with heatstroke were prospectively and consecutively enrolled, with the signed consent of their owners.

Dogs were included if collapse had occurred acutely after exposure to heat. Dogs with other coexisting medical conditions based on their history and physical examination and deemed unrelated to the heat-related illness, those for which therapy was declined by their owners, and those euthanased due to financial constraints were excluded. Thirteen staff-owned dogs, deemed healthy based on physical examination and normal complete blood count, serum biochemistry and urinalysis, were included as a negative control group for renal biomarker analysis.

Laboratory analysis

Diagnosis and severity of AKI were based on the IRIS guidelines,[1] after excluding pre- and post-renal causes. The grading used in this study was based on maximal sCr documented during hospitalization. Sera for the determination of sCr, urea and electrolytes were collected at presentation, at 4 h post-presentation and every 12 h, until discharge or death. Routine serum biochemistry and urinalysis were performed at the HUVTH Diagnostic Laboratory. Complete blood counts were performed using an automated analyzer (Advia 120, Siemens Medical Solutions, Diagnostics), as was serum biochemical analysis (Cobas-Integra 400 Plus wet chemistry analyzer, Roche, at 37 °C; Cobas B 221 electrolyte analyzer, Roche). Urine specimens were collected at presentation and stored at –80 °C, pending urinary biomarker analyses. Measurement of urine neutrophil gelatinase-associated lipocalin/creatinine ratio (UNGAL), urine C-reactive protein/creatinine ratio (UCRP) and urine retinol-binding protein/creatinine ratio (URBP) was performed with commercial ELISA kits as previously described (Nabity et al., 2012; Steinbach et al., 2014).

Urinary creatinine and Na fractional excretion (FE-Na) were measured at 4 h post-presentation, after pre-renal azotemia was corrected, to estimate the GFR and FE-Na, as previously described (Bovee and Joyce, 1979; Finco et al., 1981, 1993). For each measurement, two sequential 30 min quantitative urine collections were performed. The bladder was catheterized, emptied, and flushed three times with 10 mL sterile water at the beginning and end of each collection. Urine volume at each collection was measured and urine aliquots were stored at –80 °C, pending analysis. The creatinine and Na clearances in the two individual 30 min clearances were averaged. The fractional clearance of Na was calculated by dividing the Na clearance by the creatinine clearance.

Statistical analysis

Continuous parameters were compared between the healthy, control and heatstroke groups using Student's *t* tests or Mann–Whitney *U* tests based on data distribution as assessed using the Shapiro–Wilk test. Pearson's or Spearman's correlation tests were used, as appropriate. The receiver operator characteristics procedure, including determination of area under the curve (AUC), was used to evaluate the predictive performance of kidney function and injury parameters for the outcome (i.e. non-survival). $P \leq 0.05$ was considered statistically significant. Calculations were performed using a statistical software package (SPSS 17.0, SPSS).

Results

Thirty dogs with naturally occurring heatstroke were enrolled; median age was 4 years (range, 1–9 years) and median bodyweight was 33 kg (12–60 kg). Ten (33%) dogs were of brachycephalic breeds, including Boxer ($n = 4$), French bulldog ($n = 4$), Pug ($n = 1$) and English bulldog ($n = 1$). Twenty dogs (67%) were dolichocephalic, and the most common breeds included mixed breed ($n = 4$), Golden retriever ($n = 4$) and Labrador retriever ($n = 2$). Most dogs (23/30; 77%) presented to HUVTH between July and September. Exertional heatstroke occurred in 10/30 dogs (33%) and environmental heatstroke occurred in 20/30 (67%). The median time from onset of clinical signs to presentation was 3.8 h (0.5–14.0 h).

Median sCr at presentation was 1.69 mg/dL (range, 0.5–4.7 mg/dL; reference interval, 0.6–1.37 mg/dL), and serum sCr creatinine was ≤1.4 mg/dL in 11 dogs. After 4 h of IV fluid resuscitation for the correction of dehydration, hypovolemia and pre-renal azotemia, median sCr was 1.89 mg/dL (range, 0.6–5.6 mg/dL). Based on the IRIS grading system, 19/30 dogs (63%) developed AKI at presentation or during hospitalization. One of the 19 dogs was diagnosed with grade 1 AKI based on an increase of 0.3 mg/dL in sCr compared to the baseline concentration for that dog. Based on maximal sCr documented during hospitalization, grade 2 AKI was documented in four dogs (13%), grade 3 in 12 dogs (40%) and grade 4 in two dogs (7%). Grade 5 AKI was not diagnosed.

Thirteen dogs (43%) did not survive, leaving 17 (57%) survivors. There was no significant difference in the occurrence of AKI between survivors and non-survivors (10/13, 77% vs. 8/17, 47%, respectively; $P = 0.14$). There was no significant difference in median sCr at presentation between survivors and non-survivors (1.41 mg/dL [range, 0.73–4.74 mg/dL] vs. 2.10 mg/dL [range, 0.50–2.49 mg/dL], respectively; $P = 0.14$). However, median sCr tended to be lower at 4 h post-presentation in survivors compared to non-survivors (1.34 mg/dL [range, 0.58–4.18 mg/dL] vs. 2.50 mg/dL [range, 0.68–5.57 mg/dL], respectively; $P = 0.075$), and was significantly lower at 12 h post-presentation in survivors (1.10 mg/dL [range, 0.51–3.29 mg/dL] vs. 2.73 mg/dL [range, 0.77–4.63 mg/dL], respectively; $P = 0.049$). Serum creatinine at presentation, at 4 h and at 12 h post-presentation was a moderately accurate predictor of outcome (AUC 0.68, 95% confidence interval [CI_{95}], 0.47–0.89; AUC 0.70, CI_{95}, 0.50–0.89; AUC 0.79, CI_{95}, 0.61–0.96; respectively).

Median GFR at 4 h post-presentation was 0.60 mL/min/kg (range, 0.00–3.10 mL/min/kg). GFR was significantly higher in survivors than non-survivors (0.83 mL/min/kg [range, 0.73–3.01 mL/min/kg] vs. 0.28 mL/min/kg [range, 0.00–2.23 mL/min/kg], respectively; $P = 0.005$). GFR was <2 mL/min/kg in 23/26 dogs (88%) and <1 mL/min/kg in 18/26 dogs (69%). There was a significant inverse correlation between sCr and GFR ($r = –0.80$; $P < 0.001$). GFR was a moderate predictor of outcome (AUC 0.78, CI_{95} 0.59–0.97). The optimal cutoff point was 0.69 mL/min/kg, which yielded a sensitivity and specificity of 69% and 90%, respectively.

The median FE-Na of dogs with heatstroke at 4 h post-presentation was 0.08 (range, 0.01–0.41). Within this group, FE-Na was significantly higher in dogs with AKI grades 1–4 compared with dogs determined to be free of AKI, based on the IRIS grading system (Table 1; $P < 0.001$). There were significant moderate inverse