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Detection of indoxyl sulfate levels in dogs and cats suffering from naturally occurring kidney diseases

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ABSTRACT

Indoxyl sulfate (IS), a protein-bound uraemic toxin, has been found to accumulate in the serum of people with renal diseases and is associated with free radical induction, nephrotoxicity cardiovascular toxicity, and osteoblast cytotoxicity. Although IS has been studied in humans and in experimental models, the role of IS in dogs and cats with kidney disease has not been investigated. A high performance liquid chromatography system was applied to detect plasma IS concentrations in non-azotaemic animals (63 dogs, 16 cats) and in animals with renal azotaemia (66 dogs, 69 cats).

The IS levels of azotaemic animals were significantly higher ($P < 0.01$) than those of non-azotaemic animals (median [IQR] 20.4 (9.5) mg/L vs. 7.2 (8.8) mg/L for dogs; median [IQR] 21 (18.9) mg/L vs. 14.8 (12.3) mg/L for cats). The IS level was significantly correlated with blood urea nitrogen, serum creatinine and phosphate concentrations. Dogs with acute kidney injury had significantly higher IS levels ($P < 0.01$) than those with chronic kidney diseases (CKD) (median [IQR] 57.7 (40.8) mg/L vs. 17.7 (25.1) mg/L). When CKD was graded using the International Renal Interest Society (IRIS) staging system, IS levels were correlated with CKD severity in both dogs and cats. The IS concentration is directly related to loss of renal function. Further studies are necessary to determine whether measurement of IS provides any additional diagnostic or prognostic information in dogs and cats with kidney disease.

Introduction

Renal function is generally evaluated clinically using serum creatinine levels. However, changes in creatinine are not sufficient to accurately evaluate all uraemic syndromes nor do they show a response with every uraemic disease. Uraemic syndromes can be caused by the progressive retention of a wide range of compounds (uraemic toxins) that are normally excreted by the kidneys but accumulate when there is renal damage (Vanholder et al., 2003; Wikoff et al., 2009).

Depending on their molecular size and protein binding characteristics, uraemic toxins can be divided into three categories; water-soluble small molecules (<500 Dalton [Da]), water-soluble large molecules (≥500 Da), and protein-bound molecules (Meijers et al., 2008). Water-soluble small molecules freely pass through a dialysis membrane and therefore can be theoretically removed via dialysis therapy. However, clearance of water-soluble larger molecules, such as β2-microglobulin, and tumour necrosis factor-α, requires a high-flux dialysis membrane or ultrafiltration (Cheung et al., 2003; Chauveau et al., 2005). Notwithstanding the above, protein bound molecules including indoxyl sulfate (IS) and p-cresyl sulfate are difficult to remove by conventional haemodialysis (Lesaffer et al., 2000), and are associated with increased mortality in patients undergoing renal replacement therapy (Bammens et al., 2006).

Indoxyl sulfate is the final product of indole metabolism and has a molecular weight of 213 Da. Colonic bacteria, including *Escherichia coli*, produce tryptophanase, an enzyme that metabolizes dietary tryptophan into indole, which is then absorbed by the gut, metabolized in the liver and excreted in urine in the form of IS (Cummings, 1983; Wikoff et al., 2009). Normally around 90% of IS is bound to plasma albumin and transported into tubular cells via organic anion transporters located on the basolateral membrane of the proximal and distal tubules. Ten per cent of IS is freely filtrated by the glomerulus. In uraemic patients, due to a decrease in the glomerular filtration rate (GFR) and tubular dysfunction, IS cannot be appropriately filtered. Indoxyl sulfate has been found to induce free radicals and reduce superoxide scavenging activity, which can

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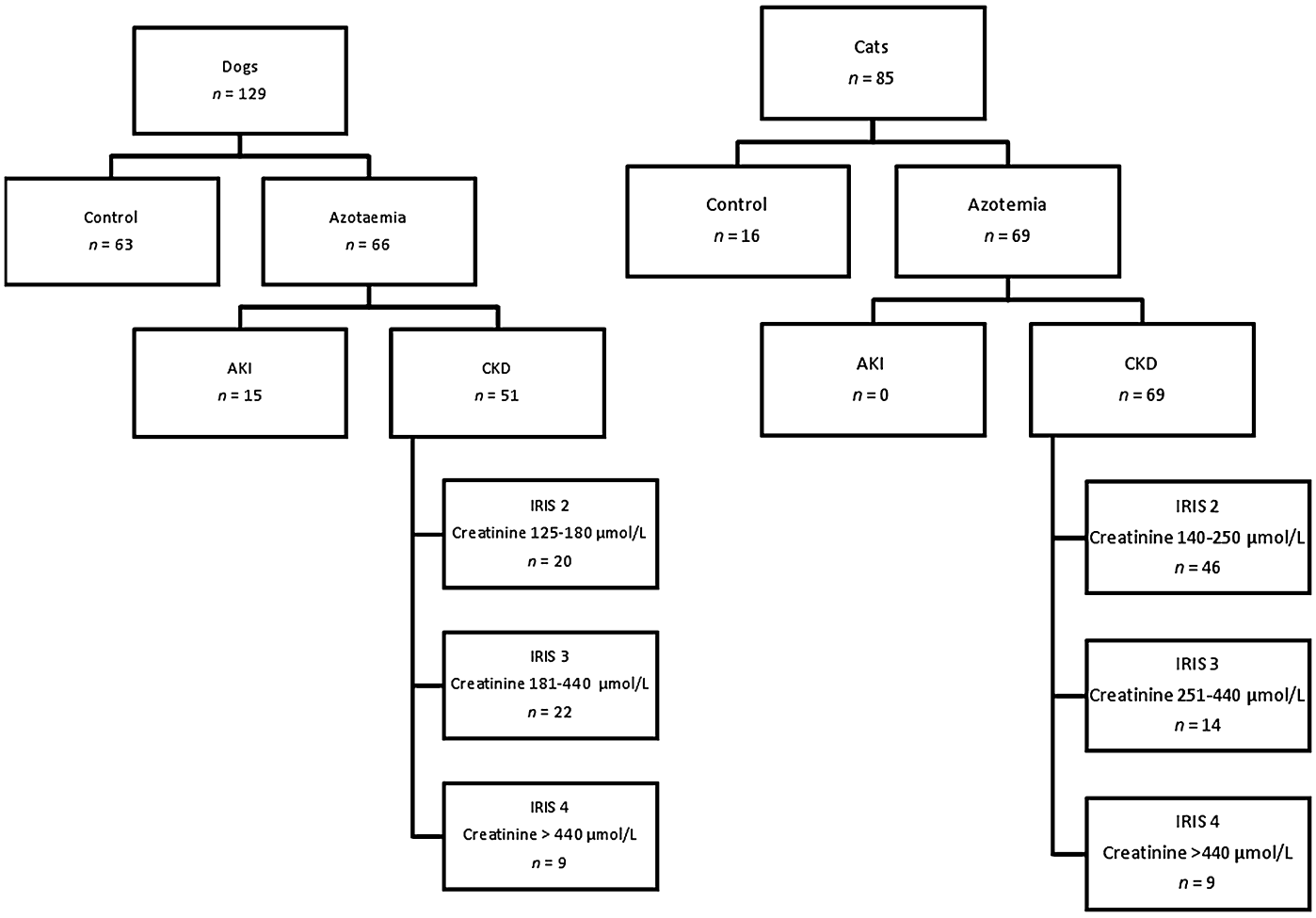

Fig. 1. Case groupings used in this study. Based on clinical parameters samples from azotaemic dogs and cats were classified as acute kidney disease (AKI) or chronic kidney disease (CKD). According to International Renal Interest Society (IRIS) stage, CKD cases were then classified into IRIS stages 2–4.

eventually result in dysfunction of the renal antioxidant system and damage renal tubular cells (Taki et al., 2006; Owada et al., 2007).

Damaged tubular cells produce transforming growth factor-b1 and other chemotactic substances such as intercellular adhesion molecule-1, monocyte chemoattractant protein-1, osteopontin and endothelin-1. These chemical substances stimulate macrophage infiltration into renal tissue, collagen production, and eventually kidney fibrosis (Niwa, 2010). Therefore, IS is not only an index of renal function but is also an aetiological agent of kidney deterioration and renal disorders. The mean concentrations of plasma IS in human uraemic patients have been reported to be higher than those of patients with normal renal function (Vanholder et al., 2003, 2014).

Indoxyl sulfate, which cannot be cleared with dialysis, is also able to inhibit endothelial cell proliferation, blunt endothelial cell repair (Dou et al., 2004), cause vascular damage (Yamamoto et al., 2006) and damage aortic smooth muscle cells (Muteliefu et al., 2009). In addition to renal toxicity, IS has been demonstrated to be associated with cardiovascular abnormalities, which can increase the mortality of patients with CKD (Barreto et al., 2009). Indoxyl sulfate has also been reported to be highly associated with atherosclerosis (Taki et al., 2007).

Although extensive studies have demonstrated the significance of IS to human renal patients (Niwa, 2010) and rat acute kidney injury (AKI) models (Fang et al., 2014), the role of IS in dogs and cats with renal disease has not been investigated to any significant extent. The aims of the current study were (1) to measure IS levels in dogs and cats with naturally occurring renal disease and (2) to evaluate the utility of IS levels as a potential indicator of renal function.

Materials and methods

Sample collection

All plasma samples were collected from dogs and cats that had been admitted to the National Chung Hsing University Veterinary Teaching Hospital including both outpatient and inpatient cases with Institutional Animal Care and Use Committee approval (103-13, February 2014).

Cases lacking clinical signs and with no history of renal disease that were presented for health examination or that had both blood urea nitrogen (BUN) and creatinine levels within reference intervals (dogs, BUN < 8.9 mmol/L, creatinine <125 µmol/L; cats, BUN < 10.7 mmol/L, creatinine < 140 µmol/L) were considered healthy and were classified as the non-azotaemic group. Animals with serum creatinine levels higher than the reference interval, excluding cases with any obstructive urinary tract disease, lower urinary disease or dehydration, were classified as the azotaemic group.

Based on patient history, cases with clinical signs for <2 weeks, or presence of oliguria (or anuria) without decreased kidney size (as confirmed by radiography/ultrasonography), were categorized as AKI. Cases with a previous history of renal disease for >1 month, or with smaller kidneys, were classified as chronic kidney disease (CKD). Based on the International Renal Interest Society (IRIS) staging system[1] (all cases were stable prior to staging), members of the CKD group were further subgrouped (Fig. 1). The management of cases with CKD mainly followed IRIS

[1] See: http://www.iris-kidney.com/guidelines/ (accessed 12 April 2015).