Research in Veterinary Science 96 (2014) 436–441

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Association between urinary vascular endothelial growth factor excretion and chronic kidney disease in hyperthyroid cats☆

T.L. Williams [a,*], J. Elliott [b], H.M. Syme [a]

[a] Department of Veterinary Clinical Sciences, Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield AL9 7TA, UK
[b] Department of Comparative Biomedical Sciences, Royal Veterinary College, Royal College Street, Camden, London NW1 0TU, UK

ARTICLE INFO

Article history:
Received 5 November 2013
Accepted 15 February 2014

Keywords:
hypoxia
PRA
NAG
RAAS
proteinuria
azotaemia

ABSTRACT

Many hyperthyroid cats develop azotaemic chronic kidney disease (aCKD) following treatment, which has led to the hypothesis that hyperthyroidism might be detrimental to renal function. Renin-angiotensin-aldosterone system (RAAS) activation occurs in hyperthyroidism, which could cause peritubular hypoxia, tubular damage and the development of aCKD. Urinary vascular endothelial growth factor:creatinine ratio (VEGFCR) is postulated to be a marker of tubular hypoxia. VEGFCR was correlated with plasma renin activity (PRA) and compared between hyperthyroid cats that did and did not develop aCKD following treatment (pre-azotaemic and non-azotaemic groups respectively). PRA was positively correlated with VEGFCR ($r_s = 0.382$; $P = 0.028$); however, pre-azotaemic hyperthyroid cats had significantly lower VEGFCR than non-azotaemic cats at baseline (median 122.3 fg/g *versus* 167.0 fg/g; $P < 0.001$). RAAS activation in hyperthyroidism is associated with increased VEGFCR; however, increased VEGFCR was not correlated with the development of aCKD. Therefore, tubular hypoxia may not be a mechanism for renal damage in hyperthyroid cats.

Hyperthyroidism and chronic kidney disease (CKD) are common comorbidities in ageing cats, with approximately 10% of hyperthyroid cats being azotaemic at the time of diagnosis of hyperthyroidism and a further 15% of hyperthyroid cats developing azotaemia within six months of treatment (Williams et al., 2010). Hence, it could be hypothesised that hyperthyroidism might be detrimental to renal function. Renin-angiotensin-aldosterone system (RAAS) activation occurs in human and feline patients with hyperthyroidism (Resnick and Laragh, 1982; Williams et al., 2013) and could cause renal damage, because angiotensin-II stimulates preferential constriction of the efferent arteriole, which may in turn lead to reduced renal peri-tubular blood flow and hypoxia of the peri-tubular tissues. In support of this, angiotensin-II receptor blockade has been demonstrated to increase interstitial microvascular oxygen tension in the renal medulla (Norman et al., 2003). The renal medulla experiences relatively low oxygen tensions under hypoxic conditions, and thus may be at high risk of hypoxic injury.

Vascular endothelial growth factor (VEGF) is a regulator of blood vessel growth, which promotes endothelial survival and helps to maintain the glomerular and peritubular vasculature in the kidney (Kang and Johnson, 2003). VEGF expression occurs in podocytes, tubular cells and mesangial cells of the human kidney (Noguchi et al., 1998; Shulman et al., 1996; Simon et al., 1995), and VEGF is produced by renal proximal tubular cells in response to hypoxia *in vitro* (El Awad et al., 2000). In human patients with preeclampsia, fractional excretion of VEGF is increased despite a relative decrease in serum VEGF concentrations, thus it has been postulated that increased renal production of VEGF occurs in pre-eclampsia (Buhimschi et al., 2006). This could indicate that hypoxia occurs in the kidney of preeclampsia patients secondary to inadequate renal perfusion. In addition, PCV is an independent predictor of urinary VEGF excretion (VEGF:creatinine ratio (VEGFCR)) in cats with azotaemic CKD.[1] Therefore, it has been suggested that increased urinary VEGFCR might be a marker of

Abbreviations: aCKD, azotaemic chronic kidney disease; ACE-I, angiotensin converting enzyme inhibitor; SBP, systolic blood pressure; CKD, chronic kidney disease; NAG, N-acetyl-β-D-glucosaminidase; NAGi, N-acetyl-β-D-glucosaminidase: creatinine ratio (NAG index); PRA, plasma renin activity; RAAS, renin-angiotensin-aldosterone system; RBP, retinol binding protein; TT4, plasma total thyroxine concentration; UAC, urine albumin:creatinine ratio; UPC, urine protein: creatinine ratio; USG, urine specific gravity; VEGF, vascular endothelial growth factor; VEGFCR, vascular endothelial growth factor:creatinine ratio

☆ Parts of this study were presented in abstract form at BSAVA Congress 2011, Birmingham, UK.

* Corresponding author. Tel.: + 7817515455; fax: + 1223339090.
E-mail address: timwilliams@cantab.net (T.L. Williams).

[1] Chakrabarti S, Syme HM, Elliott J. Urinary vascular endothelial growth factor as a marker of renal hypoxia in cats. Journal of Veterinary Internal Medicine 2011;25:720 (abstract).

http://dx.doi.org/10.1016/j.rvsc.2014.02.011
0034-5288/

renal hypoxia secondary to decreased red blood cell mass (see footnote 1). If the RAAS activation associated with hyperthyroidism leads to decreased peri-tubular blood flow and renal hypoxia, and renal hypoxia is associated with increased urinary VEGF excretion (VEGFCR), it could be postulated that plasma total thyroxine concentration (TT4) and plasma renin activity (PRA) (as a measure of RAAS activation) will correlate with urinary VEGFCR. Furthermore, VEGFCR should also correlate with other putative markers of renal injury such as urinary protein:creatinine ratio (UPC), urinary albumin:creatinine ratio (UAC) and urinary N-acetyl-β-D-glucosaminidase (NAG):creatinine ratio (or NAG index [NAGi]). Increased urinary excretion of VEGF is associated with progression of CKD and reduced survival time in cats,[2] therefore it could also be postulated that increased VEGFCR will correlate with the presence and perhaps progression of chronic kidney disease (CKD) in hyperthyroid cats.

Proteinuria is common in hyperthyroid cats, and resolves following treatment (van Hoek et al., 2009; Williams et al., 2010); however, the pathophysiological mechanism for the development of proteinuria is not currently understood. Urine protein:creatinine ratio (UPC) decreases following treatment of hyperthyroidism, whereas urine albumin:creatinine concentration (UAC) does not (Williams et al., 2010). This implies that increased urinary albumin excretion is not the cause of the increased proteinuria associated with hyperthyroidism in cats. The effect of hyperthyroidism on urinary VEGF excretion is unknown; however, it could be hypothesised that increased urinary VEGF excretion might contribute to the increased proteinuria observed in hyperthyroid cats (van Hoek et al., 2009; Williams et al., 2010).

The first aim of the present study was to investigate the associations between VEGFCR, TT4, PRA, and urinary markers of renal function such as UPC, UAC, and NAGi, in order to determine if increased VEGFCR is associated with RAAS activation and renal damage. In addition, the association between urinary VEGFCR and the development of azotaemic CKD in hyperthyroid cats following treatment was investigated. A secondary aim of the present study was to evaluate the changes in urinary VEGFCR that occur before and after treatment of hyperthyroidism, in order to determine if increased urinary VEGF excretion might contribute to the proteinuria associated with the hyperthyroid state.

1. Methods

1.1. Patient selection

Records from two London-based first opinion practices (People's Dispensary for Sick Animals, Bow and the Beaumont Sainsbury Animal Hospital, Camden) between January 1, 1999 and January 31, 2010 were reviewed and newly diagnosed hyperthyroid cats identified. Diagnosis of hyperthyroidism was based on a plasma total thyroxine concentration (TT4) greater than the laboratory reference range (>55 nmol/l, 4.3 μg/dl).

Cats included in the study were treated for hyperthyroidism with anti-thyroid medication (carbimazole or methimazole) alone, or in combination with thyroidectomy. Cats were re-examined at approximately 8- to 12-week intervals after diagnosis. Blood and urine samples were obtained at the time of diagnosis of hyperthyroidism and approximately every 4–6 months thereafter. Initially non-azotaemic hyperthyroid cats were monitored for the development of azotaemia for 120–240 days (6 ± 2 months) following documented euthyroidism. Cats were deemed to be euthyroid if they appeared euthyroid on clinical examination and had a TT4 < 40 nmol/l (3.1 μg/dl). Post treatment samples were taken at the 6 ± 2 month post treatment visit. Renal azotaemia was defined as a plasma creatinine concentration >177 μmol/l (2 mg/dl) in conjunction with inadequate urine concentrating ability (USG < 1.035), or persistent azotaemia on two or more consecutive occasions without evidence of a pre-renal cause (if urine was not available). Cats with a plasma creatinine concentration >177 μmol/l (2 mg/dl) and USG ≥ 1.035 were not classified as having renal azotaemia. Cats which had renal azotaemia at the time of diagnosis of hyperthyroidism, or which had previously been diagnosed with renal azotaemia prior to diagnosis of hyperthyroidism, were classified as '*azotaemic*', initially non-azotaemic hyperthyroid cats which developed renal azotaemia during follow up were classified as '*pre-azotaemic*', and hyperthyroid cats which remained non-azotaemic during follow up were classified as '*non-azotaemic*'.

A group of 17 non-azotaemic non-hyperthyroid (TT4 < 40 nmol/l) cats aged > 9 years old were also recruited into the study. All cats were followed for a 1-year period following sampling to ensure that azotaemia did not develop. Only cats which remained clinically healthy, non-azotaemic and euthyroid (TT4 < 40 nmol/l) during the 1-year follow-up period were included in this 'normal' ageing cat control group.

1.2. Blood and urine sampling and processing

Blood and urine samples were collected as part of a geriatric screening and healthcare programme with the consent of the owner. The Ethics and Welfare Committee of the Royal Veterinary College approved the diagnostic protocol. Jugular venous blood samples were collected and placed in EDTA coated tubes, and urine samples were collected by cystocentesis. Samples were kept at 4 °C prior to sample processing which occurred within 6 hours of sample collection. Blood samples were centrifuged at 2016 × g for 10 minutes to enable separation of plasma from cellular components. Surplus EDTA plasma was stored at –80 °C until batch analysis of PRA.

In-house urinalysis, which included measurement of urine specific gravity by refractometry, dipstick analysis and urine sediment examination, was performed on all samples. If bacteria or pyuria was identified on sediment examination, then urine was submitted for bacterial culture and sensitivity testing. Urine samples that were positive on urine culture or which were grossly haematuric were excluded from the study. Urine samples were centrifuged at 2016 × g for 10 minutes and the supernatant separated from any sediment. This was stored at –80 °C until batch analysis of urinary concentrations of total protein, albumin, VEGF and creatinine and urinary NAG activity.

1.3. Measurement of urinary concentrations of protein, albumin, VEGF and creatinine, urinary NAG activity and plasma renin activity

Urine total protein concentration was measured by a colorimetric pyrogallol red method, and urine creatinine was measured by a colorimetric picric acid method at an external laboratory.[3]

Urinary albumin concentration was measured using a sandwich ELISA as described previously (Syme et al., 2006). Calculated UPC and UAC values were previously reported as part of a larger

[2] Chakrabarti S, Syme HM, Elliott J. Urinary vascular endothelial growth factor as a prognostic marker in feline chronic kidney disease. Journal of Veterinary Internal Medicine 2012;26:1524 (abstract).

[3] Idexx Laboratories, Wetherby, UK.