Clinical evaluation of plasma aldosterone concentration and eplerenone treatment in cats and dogs with chronic kidney disease

（慢性腎臓病の猫と犬における血漿アルドステロン濃度および
エプレレノン治療の臨床学的評価）

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March 2023

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General Introduction

Aldosterone is a steroid hormone synthesized in the adrenal cortex and is part of the renin-angiotensin-aldosterone system (RAAS). It accelerates renal sodium retention and elimination of potassium through its action on the mineralocorticoid receptor (MR), and has a major role in regulating body fluid volume and blood pressure (Ponda and Hostetter, 2006). Excessive secretion of aldosterone and activation of the MR cause cardiovascular inflammation, fibrosis and remodeling, and tubulointerstitial fibrosis and glomerular injury in the kidney (Brown, 2013). There are several reports on plasma aldosterone concentration (PAC) in healthy, chronic kidney disease (CKD), systemic hypertension, and chronic heart failure in cats (Jensen *et al.*, 1997; Yu and Morris, 1998; Jepson *et al.*, 2014) and dogs (Knowlen *et al.*, 1983; Grandt *et al.*, 2022). Measurement of urinary aldosterone: creatinine ratio (UACR) has also been reported in cats (Syme *et al.*, 2007) and dogs (Lantis *et al.*, 2015; Galizzi *et al.*, 2021). However, it has been suggested that measuring aldosterone in feline urine using the available methodology has limited or no utility in investigating feline hypertension associated with kidney disease (Syme *et al.*, 2007). PAC in cats does not change significantly with age, sex, pregnancy, lactation, or circadian rhythm (Yu and Morris, 1998; Javadi *et al.*, 2004). UACR is not significantly different between healthy individuals and those with differing stages of myxomatous mitral valve disease in dogs, and is influenced by individual factors such as breed, sex and age (Galizzi *et al.*, 2021).

It may be important to evaluate PAC in dogs with CKD associated with the activation of RAAS. Plasma renin activity (PRA) is also important as a biochemical marker of RAAS activation. Although 1 study found that PRA did not significantly differ between normotensive and hypertensive cats (Jensen *et al.*, 1997), another study did show that PRA was lower in

hypertensive (both azotemic and nonazotemic) compared with control cats (Jepson *et al*., 2014). Azotemic hypertensive cats have shown significantly increased PAC and aldosterone-to-renin ratio independent of PRA (Jepson *et al*., 2014). Furthermore, PRA in cats with reduced renal functions is reportedly less sensitive to salt intake than PAC (Buranakarl *et al*., 2004). This event may be advantage on measurement of PAC over PRA. PRA also differs with age and sex/neuter status in cats (Javadi *et al*., 2004). These findings propose the significance of measuring PAC in hypertensive CKD cats.

Angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) suppress the RAAS during hypertensive, renal, and cardiac diseases in cats (Jensen *et al*., 1997; Glaus *et al*., 2019; Ames *et al*., 2019; Coleman *et al*., 2019; Steele *et al*., 2002). The Randomized Aldactone Evaluation Study (RALES) conducted in humans showed that the aldosterone antagonist, spironolactone, reduced the mortality of patients with chronic heart failure who received ACEI and loop diuretics by 30% (Pitt *et al*., 1999). Spironolactone also reportedly reduced the mortality rate in cats with congestive heart failure secondary to cardiomyopathy (James *et al*., 2018), but the authors were cautious in concluding this as the cats in the treatment group appeared to have less severe disease than the placebo group. Another selective aldosterone antagonist, eplerenone, not only antagonizes MR (Delyani *et al*., 2001) but also blocks the nongenomic effects of aldosterone in vascular tissues not susceptible to spironolactone (Losel *et al*., 2003; Michea *et al*., 2005). These effects of eplerenone might be more effective than spironolactone in treating hypertension due to vasoconstriction. However, the clinical significance of this effect is still unclear in dogs and cats. Furthermore, although eplerenone reduces mortality and hospitalization in human patients with chronic heart failure (Zannad *et al*., 2011; Ferreira *et al*., 2019), there are no available reports on eplerenone's use in

feline practice. Hence, spironolactone is the only MR antagonist currently reported for its effect in cat-based clinical trials (MacDonald *et al*., 2008; James *et al*., 2018; Spencer *et al*., 2020).

The author hypothesized that if an elevated PAC is detectable in the early stages of the disease in cats, the use of mineralocorticoid antagonists may prolong lifespan. Elevated PAC is a risk factor for kidney injury in humans, and MR antagonists are beneficial in rodent models of CKD and human patients (Spencer *et al*., 2020). However, the relationship between PAC and the survival time in cats and dogs with CKD has not been investigated. Therefore, this study was conducted to investigate PAC in cats and dogs with CKD, and evaluate the influence of high PAC on the survival time of CKD animals and the effect of treatment with eplerenone in CKD cats with high PAC.

In chapter 1, the study aimed to investigate PAC in cats with CKD, and evaluate the survival of cats with high PAC. Furthermore, the effect of treatment with eplerenone on the survival time in CKD cats with high PAC was examined. The eplerenone study was conducted including both cats with CKD only and CKD cats complicated cardiac disease or systemic hypertension. In chapter 2, the study aimed to investigate PAC in dogs with CKD, and evaluate the survival of CKD dogs with high PAC.

Chapter 1

Effects of plasma aldosterone concentration and treatment with eplerenone on the survival of cats with chronic kidney disease

Introduction

Aldosterone is a steroid hormone synthesized in the adrenal cortex and is part of the renin-angiotensin-aldosterone system (RAAS). It accelerates renal sodium retention and elimination of potassium through its action on the mineralocorticoid receptor (MR), and has a major role in regulating body fluid volume and blood pressure (Ponda and Hostetter, 2006). The MR is also present in other tissues besides the kidney, including cardiomyocytes and vascular endothelial cells. Aldosterone is locally produced in the vasculature, kidney, and heart in addition to the adrenal gland (Weber *et al*., 2003), and its actions may induce classical genomic, as well as rapid nongenomic effects (Brown, 2013). Nongenomic effects of aldosterone are proposed to potentiate angiotensin II-induced vasoconstriction and facilitate classical MR-mediated effects (Weber *et al*., 2003; Brown, 2013). In rodents, excessive secretion of aldosterone and activation of the MR cause cardiovascular inflammation, fibrosis and remodeling, and tubulointerstitial fibrosis and glomerular injury in the kidney (Brown, 2013).

There are several reports on plasma aldosterone concentration (PAC) in cats that are healthy and in those with chronic kidney disease (CKD) and systemic hypertension (Jensen *et al*., 1997; Yu and Morris, 1998; Jepson *et al*., 2014). Measurement of urinary aldosterone:creatinine ratio has also been reported in cats (Syme *et al*., 2007). However, it has been suggested that measuring aldosterone in feline urine using the available methodology has limited or no utility in investigating feline hypertension associated with kidney disease (Syme *et al*., 2007). The PAC in cats does not change significantly with age, sex, pregnancy, lactation, or circadian rhythm (Yu and Morris, 1998; Javadi *et al*., 2004). Plasma renin activity (PRA) is also important as a biochemical marker of RAAS activation. Although 1 study reported PRA did not significantly

differ between normotensive and hypertensive cats (Jensen *et al*., 1997), another study reported that PRA was lower in hypertensive (both azotemic and nonazotemic) compared to control cats (Jepson *et al*., 2014). Azotemic hypertensive cats have a significantly increased PAC and aldosterone-to-renin ratio independent of plasma renin activity (Jepson *et al*., 2014). Furthermore, PRA in cats with reduced renal functions is reportedly less sensitive to salt intake than PAC (Buranakarl et al., 2004). The PRA also differs with age and sex/neuter status (Javadi et al., 2004). These findings propose the significance of measuring PAC in hypertensive CKD cats. However, PAC varies with dietary sodium and potassium intake (Dow *et al*., 1990; Yu *et al*., 1997; Yu and Morris, 1997; Reynolds *et al*., 2013; Buranakarl *et al*., 2004) and a renal diet (sodium restriction) may be expected to elevate the PAC in cats. The RAAS is activated in cats with reduced renal function at the lowest salt intake and is associated with hypokalemia and a high excretion of potassium (Buranakarl *et al*., 2004).

Angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) suppress the RAAS during hypertensive, renal, and cardiac diseases in cats (Jensen *et al*., 1997; Steel *et al*., 2002; Ames *et al*., 2019; Coleman *et al*., 2019; Glaus *et al*., 2019). In the Randomized Aldactone Evaluation Study (RALES) conducted in humans, the MR antagonist, spironolactone, reduced mortality (by 30%) of patients with chronic heart failure that received ACEI and loop diuretics (Pitt *et al*.,1999). Spironolactone also reportedly reduced the mortality rate in cats with congestive heart failure secondary to cardiomyopathy (James *et al*., 2018), but the authors were cautious in concluding this, as cats in the treatment group appeared to have less severe disease than the placebo group. Another selective MR antagonist, eplerenone, not only antagonizes MR (Delyani *et al*., 2001) but also blocks the nongenomic effects of aldosterone in vascular tissues not susceptible to spironolactone (Losel *et al*., 2003; Michea *et al*., 2005). These

effects of eplerenone might be more useful than spironolactone in treating hypertension due to vasoconstriction. However, the clinical relevance of this effect is still unclear in dogs and cats. Furthermore, although eplerenone reduces mortality and hospitalization in human patients with chronic heart failure (Zannad *et al*., 2011; Ferraira *et al*., 2019), there are no available reports on eplerenone's use in feline practice. Hence, spironolactone is the only MR antagonist currently reported for its effect in cat-based clinical trials (MacDonald *et al*., 2008; James *et al*., 2018; Spencer *et al*., 2020).

We hypothesized that if an elevated PAC is detectable in the early stages of CKD in cats, the use of MR antagonists may prolong lifespan. Although elevated PAC is a risk factor for kidney injury in humans, MR antagonists are beneficial in rodent models of CKD and human patients (Spencer *et al*., 2020). However, the relationship between PAC and survival rate in CKD has not been investigated in cats. Therefore, this study investigated PAC in cats with CKD and evaluated survival of cats with high PAC. Furthermore, we examined the effect of eplerenone on survival time in CKD cats with high PAC.

Materials and methods

Animals

Records from client-owned cats that visited the Yasaka Animal Care Center, Japan, between October 2016 and December 2021 and that had stored blood samples available, were reviewed retrospectively. This hospital provided general practice for local clients and referral services for veterinarians throughout the local area. Cats (N = 156) were identified with the diseases of interest including CKD, cardiac disease and systemic hypertension, and categorized into the following groups: clinically healthy (n = 101) and CKD (n = 55). Cats diagnosed with other concurrent disease were excluded.

Grouping

Blood samples were collected from healthy cats and cats diagnosed with CKD by routine diagnostic procedures. Clinically healthy cats visited the hospital for routine health examinations, had no signs of illness, and received no medication. Subsequently, physical examination, hematological and biochemical examinations including blood urea nitrogen (BUN), creatinine, aspartate aminotransferase, alanine aminotransferase, glucose, and total protein and systemic blood pressure measurement were conducted for the health check. Informed consent was obtained for the use of the blood samples.

Hematological and biochemical examinations, urinalysis, blood pressure measurement, radiography, and abdominal ultrasonography were used to diagnose CKD. Urine protein/creatinine ratio was examined when urinary protein was detected on dipstick. When bacteria were detected in the urine sediment, urine culture was performed for antibiotic susceptibility test with disk diffusion method. Cats with CKD were subsequently classified using

the International Renal Interest Society (IRIS) staging of CKD (modified 2019). Cats with a temporary and rapid increase in plasma creatinine exceeding the reference value, as occurs in acute kidney injury, were excluded from the study. Cats with hyperthyroidism were also excluded. Cats with kidney diseases including polycystic kidney disease, chronic urinary tract obstruction, chronic urinary tract infection, urolithiasis, feline urological syndrome, and renal lymphoma, were included in the group. None of the cats was suspected of having toxic nephropathy. In cases such as chronic urinary tract obstruction and urinary tract infection, when the disease was treated and remitted, cats having chronic renal dysfunction on subsequent follow-up were included in the CKD group. The number of cats in IRIS Stages 1, 2, 3, and 4 were 0, 32, 18, and 5, respectively. Clinical signs in IRIS Stage 2 or higher included polyuria, oliguria, anuria, weight loss, dehydration, anemia, respiration abnormality, diarrhea, emesis, lethargy, or depression. Since this study mainly investigated the significance of PAC measurements associated with CKD, cats with primary hyperaldosteronism were excluded. Cats with primary hyperaldosteronism due to an adrenocortical tumor were excluded by abdominal ultrasonography.

Physical examination, hematological and biochemical examinations, electrocardiogram, blood pressure measurements, radiography, and echocardiography were used to diagnose cats with cardiac disease. A veterinary cardiologist certified by the Japanese Society of Veterinary Cardiology or a veterinarian with considerable experience and further training in cardiology conducted echocardiography.

Hypertension was diagnosed through non-invasive measurements using a Doppler or oscillometric device. Doppler was routinely used unless the cats were poorly tolerant, in which case oscillometric measurement was employed. After each cat was rested in a separate room,

their blood pressure was measured multiple times until it became stable, and the average value was calculated from 5 to 10 stable measurements. Systolic blood pressure (SBP) of ≥ 160 mmHg was defined as "hypertensive" based on the American College of Veterinary Internal Medicine (ACVIM) consensus statement on hypertension (Acierno *et al*., 2018). Blood pressure was measured routinely in all cats with CKD and cardiac disease. It was also routinely measured in healthy cats for health examination, and if their SBP was < 160 mmHg, it was recorded as non-hypertension.

Most cats were receiving no medication at the time of hospital admission, but some cats had received treatment by the time blood sampling was performed. Eight cats in the CKD group received benazepril or amlodipine. Some cats had > 1 blood sample available to measure PAC. Therefore, PAC values during health examination in the healthy group and at diagnosis in the diseased group were used to compare PACs between the groups.

Sample processing and analysis of PAC

Blood was mixed with EDTA and immediately centrifuged at room temperature, with plasma separated and frozen at –35°C until PAC analysis. Concentrations of PAC were measured by solid-phase radioimmunoassay (RIA), using a kit (SPAC-S Aldosterone Kit, Fujirebio, Tokyo, Japan). The RIA method in the kit used was the same principle as previously validated for use with feline plasma in a commercially available human kit (Jepson *et al*., 2014). Intra- and inter-assay coefficients of variation were 1.8 to 8.3% and 2.4 to 3.2%, respectively. The kit was validated for use in cats by adding 2 ranges of aldosterone control (53 to 88 pg/mL and 252 to 420 pg/mL) extracted from the human matrix to feline plasma. Lower and upper detection limits were 10 and 1600 pg/mL, respectively.

Determination of PAC reference range

The reference range for PAC was determined at a 95% confidence interval (CI) by a nonparametric statistic method using values from 101 healthy cats. With a median PAC of 89 pg/mL, the lower limit was 10 pg/mL at 5% percentile, and the upper limit was 243 pg/mL at 95% percentile. Therefore, the reference range for normal PAC was defined as 10 to 243 pg/mL, with PAC exceeding the upper limit defined as "high" PAC.

Survival rates of animals with high plasma aldosterone concentration (PAC) and effect of eplerenone

Survival days were calculated from the blood sampling date to compare long-term outcomes between high and normal PAC levels in the CKD group.

The eplerenone study was conducted prospectively on cats with higher PAC than the reference value. Eight cats were used to examine the effect of eplerenone on the survival time of cats with CKD and a high PAC. Seven of these 8 cats had concurrent disease, including heart disease and/or arterial hypertension. Eighteen CKD cats with a high PAC were used as the non-eplerenone control group. Eight of these 18 had concurrent disease, including heart disease and/or arterial hypertension. Cats in the non-eplerenone group had similar clinical characteristics and biochemical variables to those in the eplerenone-treated group. Since no previous studies using eplerenone in cats were identified, informed consent was obtained from each cat owner regarding eplerenone administration. Owners and clinicians were not blinded to the eplerenone treatment (or not) group. The cats were alternately assigned to either the control group or eplerenone treatment group, in the order they visited the hospital. Informed consent was sought

to owners regarding eplerenone administration, but some owners did not accept it. The reason for non-acceptance was that there was no report on eplerenone medication in cats. Therefore, the number of cats was greater in the non-eplerenone group than the eplerenone group. Eplerenone was orally administered at 2.5 to 5 mg/kg body weight (BW) once daily, based on previous dosages reported to have been effectively and safely used in dogs (Arita *et al*., 2020). Ethical approval was not required for the eplerenone study; however, informed consent was essential for inclusion. Veterinarians were responsible for any adverse reactions that may occur as a result of this unlicensed use for cats. They were also required to retain medical records including the name of the formulation, dosage, and manufacturer for at least 3 y.

Statistical analysis

Data were analyzed using statistical software (Prism 7.0, GraphPad, California, USA). Difference in sex frequencies between groups was compared using Fisher's Exact test. Data including PAC, age, SBP, and biochemical variables, were tested for normality using the Shapiro–Wilk test. When the data were abnormally distributed, these nonparametric data were subjected to the Kruskal–Wallis test. When significant *P* values were encountered, the *post-hoc* Dunn's multiple comparison test was used to determine significant differences between the groups. One-way analysis of variance (ANOVA) and *post-hoc* Tukey's multiple comparison test were used for intergroup comparisons when the data were normally distributed. The Mann–Whitney test or unpaired Student's *t*-test was used to compare data between 2 groups to determine the difference. Kaplan–Meier curves were constructed to compare survival rates, and log-rank (Mantel-Cox) tests were used to compare survival curves because the curves were right-

skewed and censored. The hazard ratio was expressed as 95% CIs. The significance level for each analysis was at $P < 0.05$.

Results

Age, sex, breed, blood biochemistry, SBP, and diagnosis of cats in the healthy and CKD groups are summarized in Table 1. Age [median (minimum–maximum)] in the healthy and CKD groups were 0.8 (0.3–16.2) and 15.3 (1.7–23.1) years, respectively. Cats in the healthy group were younger ($P < 0.01$, by Mann–Whitney test) than those in the CKD group.

Comparison of PAC between healthy and CKD groups

The PAC in the healthy and CKD groups was median (minimum–maximum); 89 (10–416) and 126 (10–981) pg/mL, respectively (Table 1; Figure 1). Linear regression analysis observed no significant correlation (R squared = 0.00148; $P = 0.70$) between PAC and age in the healthy group (Figure 2). PAC in the CKD group was higher ($P < 0.01$, by Mann–Whitney test) than the healthy group (Figure 1). Since cats in the clinically healthy group were significantly younger than those in the CKD group, age-matched control data for PAC analysis were pulled from the healthy group. The PAC, plasma creatinine, and BUN values in the age-matched control (≥ 10 y old; age 12.6 ± 1.9 (mean ± SD), $n = 12$) and younger healthy cats (< 10 y old; age 2.1 ± 2.8, $n = 89$) were 85 ± 53 and 98 ± 68 pg/mL, 1.3 ± 0.2 and 1.2 ± 0.2 mg/dL, and 24.5 ± 5.0 and 23.9 ± 3.3 mg/dL, respectively. There were no significant differences ($P = 0.72$, 0.15, and 0.56, respectively; by Mann–Whitney test or unpaired t-test) in those values between young-healthy cats and old-healthy cats age-matched to CKD cats. The PAC was greater ($P = 0.03$, by Mann–Whitney test) in CKD cats than in age-matched healthy cats.

Blood biochemistry and SBP in cats in the CKD group as classified by IRIS stage are summarized in Table 2. The PACs were higher ($P = 0.03$, Kruskal–Wallis test followed by

Dunn's multiple comparison test) in IRIS stage 2 than in the healthy group (Figure 3). Similarly, PACs in IRIS stage 3 and 4 cats were higher ($P = 0.01$) than in the healthy group. At IRIS Stages 3 and 4, individual differences in PACs were great. In particular, IRIS Stage 4 cats ($n = 5$) had a large variation in PAC (median = 98 pg/mL; minimum–maximum = 10–981 pg/mL).

Survival analysis in cats with high vs. normal PAC

Blood biochemistry, SBP and treatments of cats with normal and high PAC used for evaluating the survival time in CKD group are shown in Table 3. In the CKD group, cats with high PAC had shorter ($P = 0.019$, by log-rank test) survival periods than those with normal PAC (Figure 4). Median (minimum–maximum) survival of cats with high PAC and normal PAC was 446 days (29–1586 days) and 1233 days (11–1850 days), respectively. Hazard ratio (high/normal PAC) for risk of death was 2.21. On day 0, normal and high PACs [median (minimum–maximum)] were 94 pg/mL (10–222 pg/mL) and 307 pg/mL (250–981 pg/mL), respectively. There were no significant differences in age, plasma creatinine, BUN, and SBP values between normal and high PAC groups (Table 3). Treatments and IRIS stages between normal and high PAC groups were similar (Table 4).

Effects of eplerenone on survival in cats with high PAC

Age, sex, diagnosis, blood biochemistry, SBP, and medications of the eplerenone and non-eplerenone treatment groups in high PAC cats are shown in Table 5. In cats with high PAC and CKD, eplerenone administration prolonged ($P = 0.005$, by log-rank test) survival compared to cats not receiving eplerenone (Figure 5). Median (minimum–maximum) survival of cats with eplerenone and non-eplerenone was 1109 days (333–1391 days) and 243 days (17–1312 days),

respectively. Hazard ratio (eplerenone/non-eplerenone) was 0.35. The PACs [median (minimum-maximum)] on day 0 in cats receiving and not receiving eplerenone were 356 pg/mL (269–540 pg/mL) and 338 pg/mL (252–548 pg/mL), respectively. There was no significant difference between the groups in age, PAC, plasma creatinine, BUN, or SBP. Treatments, IRIS stages, and complications were similar between groups. There was no significant difference in median BUN values between the groups, but the non-eplerenone group included 3 cats with extremely high BUN values (range: 88 to 127 mg/dL), whereas the eplerenone group included no cats with such high BUN values (maximum 46 mg/dL). When the cause of death was judged clinically, deaths in the non-eplerenone group were due to natural or sudden cause in 13 cats, heart failure in 5 cats, and renal failure in 2 cats. Deaths in the eplerenone group were due to natural cause in 1 cat, heart failure in 3 cats, and renal failure in 4 cats.

Table 1. Age, sex, breed, blood biochemistry, systemic blood pressure and diagnosis of cats in the healthy and chronic kidney disease (CKD) groups.

Variables	Healthy	CKD
Number of cats	101	55
Age (y)[a]	0.8 (0.3–16.2)	15.3 (1.7–23.1)*
Male/Female (*n*)	50/51	23/32
(Castrated/Ovariohysterectomized)	(16/19)	(21/32)
Breed (*n*)	Domestic shorthairs (92) Norwegian Forest Cats (3) Scottish Folds (3) Himalayan Cats (2) Russian Blues (1)	Domestic shorthairs (49) Abyssinians (2) Norwegian Forest Cats (1) Scottish Folds (1) Russian Blues (1) American Shorthairs (1)
Diagnosis (*n*)	None (101)	IRIS-2 (32) IRIS-3 (18) IRIS-4 (5)
Blood urea nitrogen (mg/dL)[a]	24 (14–32)	36 (18–140)*
Plasma creatinine (mg/dL)[a]	1.2 (0.6–1.5)	2.6 (1.7–9.6)*
Systolic blood pressure (mmHg)[b]	<160	140 ± 10
Plasma aldosterone concentration (PAC) (pg/mL)[a]	89 (10–416)	126 (10–981)*

[a] Median (minimum–maximum).

[b] Mean ± standard deviation.

* $P < 0.01$, different from the healthy group.

Table 2. Blood biochemistry and systemic blood pressure in cats in the chronic kidney disease (CKD) group as classified by IRIS stage.

Variables	Healthy	IRIS-2	IRIS-3 and 4
Number of cats	101	32	23
Age (y)[a]	0.8 (0.3–16.2)	13.9 (4.0–23.1)**	17.4 (1.7–20.8)**
Male/female (*n*) (Castrated/ovariohysterectomized)	50/51 (16/19)	16/16 (15/16)	7/16 (6/16)
Blood urea nitrogen (mg/dL)[a]	24 (14–32)	29 (18–48)**	57 (25–140)** ††
Plasma creatinine (mg/dL)[a]	1.2 (0.6–1.5)	2.2 (1.7–2.8)**	3.7 (2.9–9.6)**†
Systolic blood pressure (mmHg)[b]	< 160	139 ± 10	142 ± 10
Plasma aldosterone concentration (PAC) (pg/mL)[a]	89 (10–416)	123 (14–443)*	148 (10–961)*

IRIS – International Renal Interest Society.

[a] Median (minimum–maximum).

[b] Mean ± standard deviation.

* $P < 0.05$, ** $P < 0.01$, different from healthy group.

† $P < 0.05$, †† $P < 0.01$, different from IRIS-2 group.

Table 3. Blood biochemistry, systemic blood pressure and medications of cats with normal and high PAC used for survival analysis in chronic kidney disease (CKD) group.

Variables	Normal PAC	High PAC
Number of cats	39	16
Age (y)[a]	14.2 (1.7–23.1)	16.1 (7.3–20.8)
Male/female (*n*) (Castrated/ovariohysterectomized)	20/19 (18/19)	3/13* (3/13)
IRIS stage (% incidence)	IRIS-2 (61%) IRIS-3 (31%) IRIS-4 (8%)	IRIS-2 (50%) IRIS-3 (38%) IRIS-4 (12%)
Plasma aldosterone concentration (PAC) (pg/mL)[a]	94 (10–222)	307 (250–981)**
Blood urea nitrogen (mg/dL)[a]	34 (18–119)	42 (18–140)
Plasma creatinine (mg/dL)[a]	2.6 (1.7–9.6)	3.0 (2.1–8.0)
Systolic blood pressure (mmHg)[b]	140 ± 8	141 ± 15
Treatment (n)	Kidney support diet (14)[c], Fluid infusion (8), Benazepril 0.5-0.6 mg/kg, SID (4), Amlodipine 0.2-0.4 mg/kg, SID or BID (1), Potassium gluconate (2), Phosphate binder (2), or Antibiotics (1)	Kidney support diet (9)[c], Fluid infusion (4), Benazepril 0.5-0.6 mg/kg, SID (4), Amlodipine 0.2-0.4 mg/kg, SID or BID (3), Potassium gluconate (2), or Phosphate binder (1)
Survival time (d)[a]	1233 (11–1850)	446 (29–1586)*

PAC – Plasma aldosterone concentration; IRIS – International Renal Interest Society.

[a] Median (minimum–maximum).

[b] Mean ± standard deviation.

[c] Specific diet formulated with low phosphorous and a moderate level of highly digestible protein (Royal Canin, Renal with chicken). Analytical constituents are as follows: protein – 8%; fat content – 8%; crude ash – 1.3%; crude fiber – 0.8%; moisture – 77%; calcium – 0.15%; phosphorus – 0.08%; potassium – 0.2%; sodium – 0.11%; magnesium – 0.015%; iron – 0.003%; copper – 0.0005%; zinc – 0.003%; EPA and DHA – 0.15%; taurine – 0.14%; arginine – 0.4%; vitamin E – 0.015%; vitamin C – 0.007%; vitamins B – 0.007%; and others.

* $P < 0.05$, ** $P < 0.01$, different from normal PAC.

Table 4. Medications of cats with normal and high plasma aldosterone concentration (PAC) used for survival analysis in chronic kidney disease (CKD) group.

Group	IRIS stage (*n*)	Treatment (*n*)
Normal PAC	IRIS-2 (24)	None (20)
		Kidney support diet (2)
		Benazepril 0.5-0.6 mg/kg, SID (2)
		Fluid infusion (1)
	IRIS-3 (12)	None (2)
		Kidney support diet (10)
		Fluid infusion (5)
		Benazepril 0.5-0.6 mg/kg, SID (2)
		Potassium gluconate (2)
		Phosphate binder (2)
		Amlodipine 0.2-0.4 mg/kg, SID or BID (1)
	IRIS-4 (3)	Kidney support diet (2)
		Fluid infusion (2)
		Antibiotics (1)
High PAC	IRIS-2 (8)	None (5)
		Kidney support diet (3)
		Benazepril 0.6 mg/kg, SID (2)
		Amlodipine 0.2 mg/kg, SID (1)
		Potassium gluconate (1)
		Fluid infusion (1)
	IRIS-3 (6)	Kidney support diet (5)
		Benazepril 0.6 mg/kg, SID (2)

	Amlodipine 0.2-0.4 mg/kg, SID (2)
	Potassium gluconate (1)
	Phosphate binder (1)
	Fluid infusion (2)
IRIS-4 (2)	Kidney support diet (1)
	Fluid infusion (1)

IRIS – International Renal Interest Society.

Table 5. Age, sex, diagnosis, blood biochemistry, systemic blood pressure and medications of eplerenone and non-eplerenone treatment groups in high PAC cats.

Variables	Non-eplerenone	Eplerenone
Number of cats	18	8
Age (y)[a]	16.6 ± 3.4	15.0 ± 1.8
Male/Female (*n*) (Castrated/Ovariohysterectomized)	8/10 (8/9)	3/5 (3/4)
IRIS stage (*n*)	IRIS-2 (9), IRIS-3 (9)	IRIS-2 (5), IRIS-3 (3)
Diagnosis (*n*)	CKD (10) CKD and hyperthyroidism (1) CKD and HCM (CHF) (2) CKD and hypertension (3) CKD, hyperthyroidism and hypertension (1) CKD, HCM (CHF) and hypertension (1)	CKD (1) CKD and MI (1) CKD and hypertension (2) CKD and HCM (CHF) (1) CKD and RCM (CHF) (1) CKD, MI and hypertension (1) CKD, HCM (CHF) and hypertension (1)
Plasma aldosterone concentration (PAC) (pg/mL)[a]	360 ± 95	381 ± 100
Blood urea nitrogen (mg/dL)[b]	42 (19–127)	34 (26–46)
Plasma creatinine (mg/dL)[a]	2.9 ± 0.9	2.8 ± 0.6
Systolic blood pressure (mmHg)[a]	164 ± 32	167 ± 22

Treatment	Kidney support diet[c], Fluid infusion, Benazepril (0.5-0.6 mg/kg BW, PO, q24h), Amlodipine (0.2-0.4 mg/kg BW, PO, q12h or q24h), Pimobendan (0.2-0.3 mg/kg BW, PO, q12h), Methimazole (0.34-0.54 mg/kg BW, PO, q12h or q24h), Furosemide (0.5-1 mg/kg BW, PO, q12h), Potassium gluconate, or Phosphate binder	Kidney support diet[c], Fluid infusion, Benazepril (0.5-0.6 mg/kg BW, PO, q12h or q24h), Amlodipine (0.22 mg/kg BW, q24h), Pimobendan (0.26-0.3 mg/kg BW, PO, q12h), or Furosemide (1-2 mg/kg BW, PO, q12h or q24h)
Survival time (day)[b]	243 (17–1312)	1109 (333–1391)*

PAC – Plasma aldosterone concentration; IRIS – International Renal Interest Society; CKD – Chronic kidney disease; HCM – Hypertrophic cardiomyopathy; CHF – Congestive heart failure; MI – Mitral insufficiency; RCM – Restrictive cardiomyopathy; BW – Body weight.

[a] Mean ± standard deviation.

[b] Median (minimum–maximum).

[c] Specific diet formulated with low phosphorous and a moderate level of highly digestible protein (Royal Canin, Renal with chicken).

* $P < 0.01$, different from non-eplerenone group.

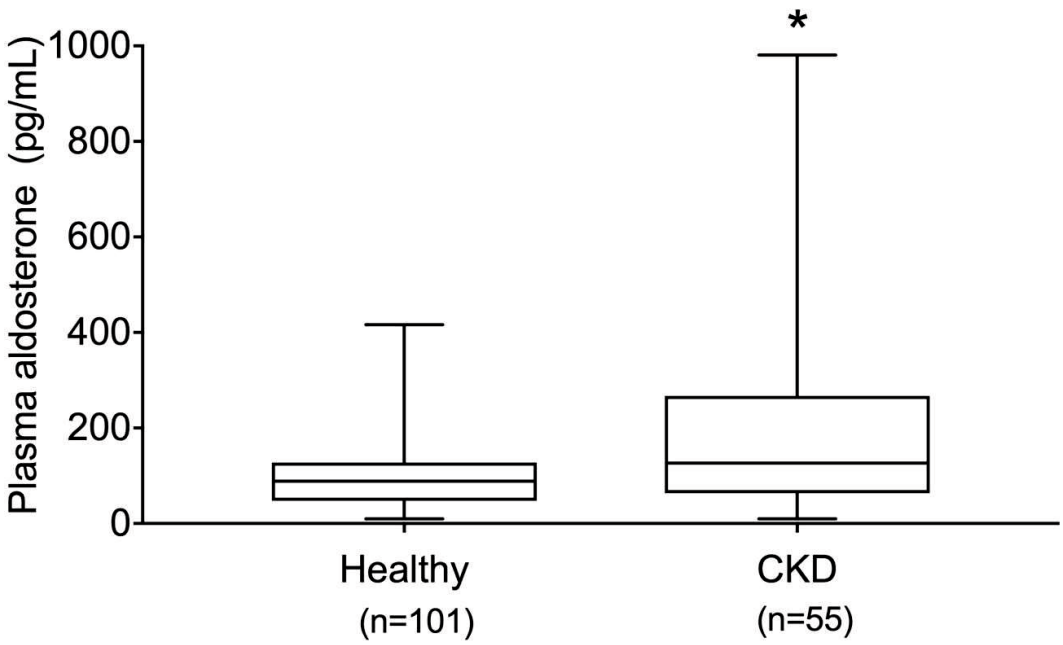

Figure 1. Plasma aldosterone concentration in healthy (n = 101) and chronic kidney disease (CKD) (n = 55) groups. The boxes represent the 25th and 75th quartiles, with the horizontal line representing the median. The whiskers represent the data range. * $P < 0.01$, showed significant difference from the healthy group by the Mann–Whitney test.

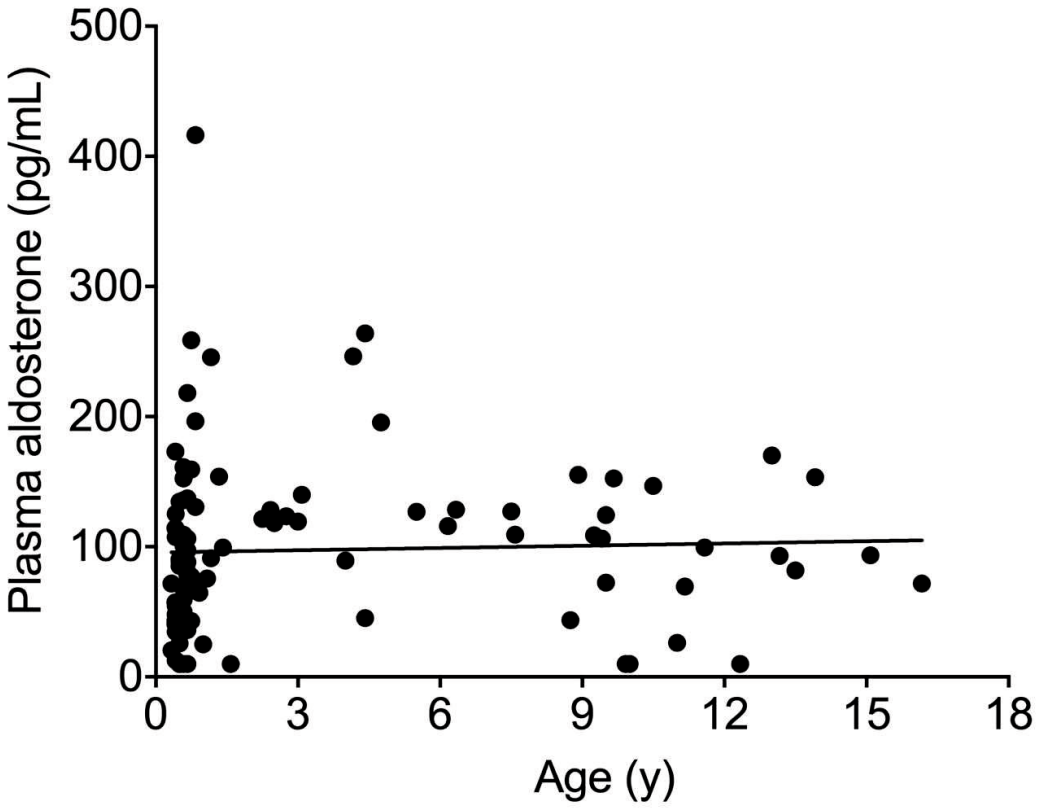

Figure 2. Correlation between plasma aldosterone concentration and age in 101 healthy cats. Data were analysed using linear regression analysis.

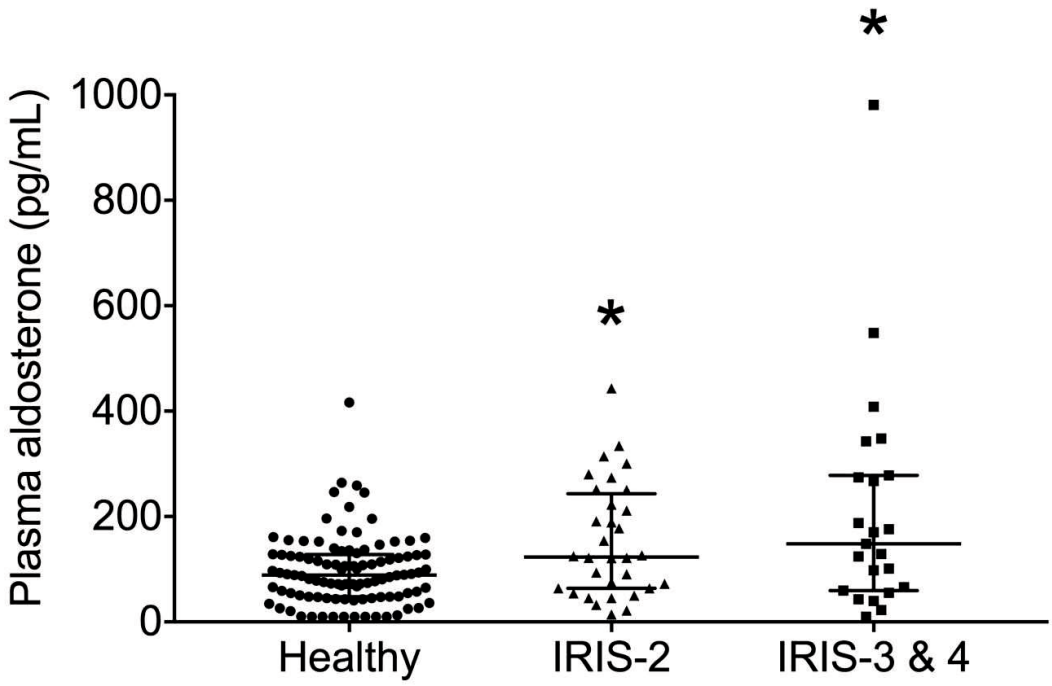

Figure 3. Plasma aldosterone concentrations in healthy (n = 101), IRIS stage 2 (n = 32), and IRIS stage 3 (n = 18) and 4 (n = 5) cat groups. The bars and whiskers indicate the median, 25th, and 75th quartiles. * $P < 0.05$, showed significant difference from the healthy group by the Kruskal–Wallis test, followed by Dunn's multiple comparison test.

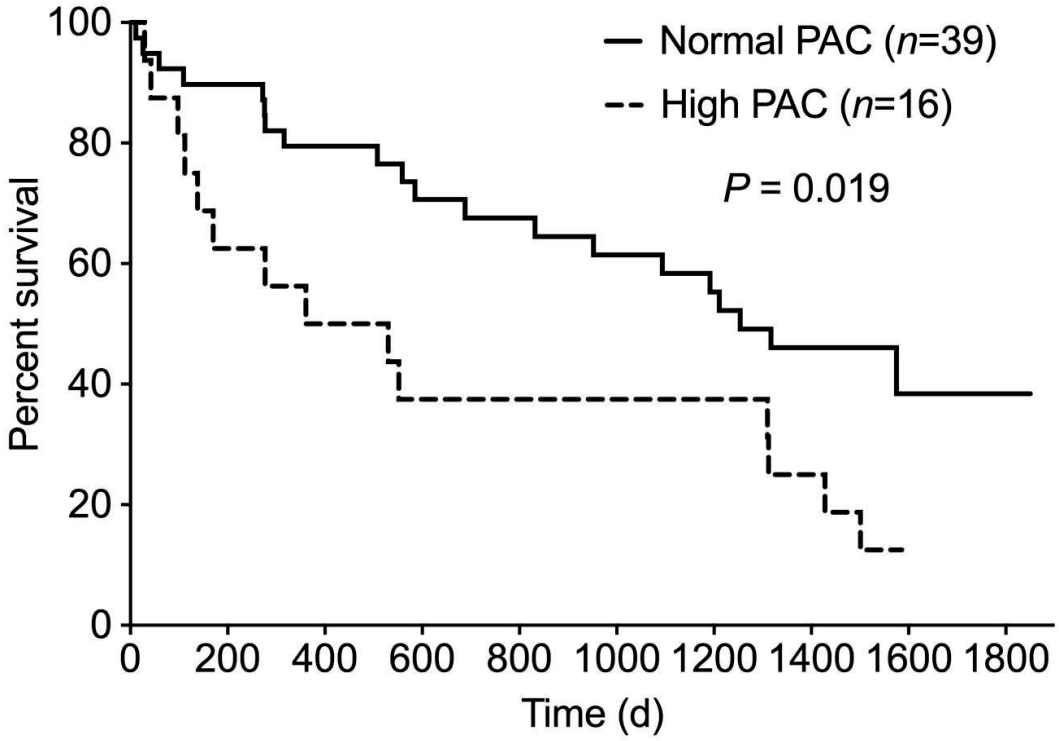

Figure 4. Kaplan–Meier survival curves comparing survival time of cats with normal (solid line) and high (dashed line) plasma aldosterone concentration (PAC) in the chronic kidney disease (CKD) group. While high PAC was set at > 243 pg/mL, normal PAC was 10–243 pg/mL. *P*-value, showing statistically significant differences, is presented above the graph.

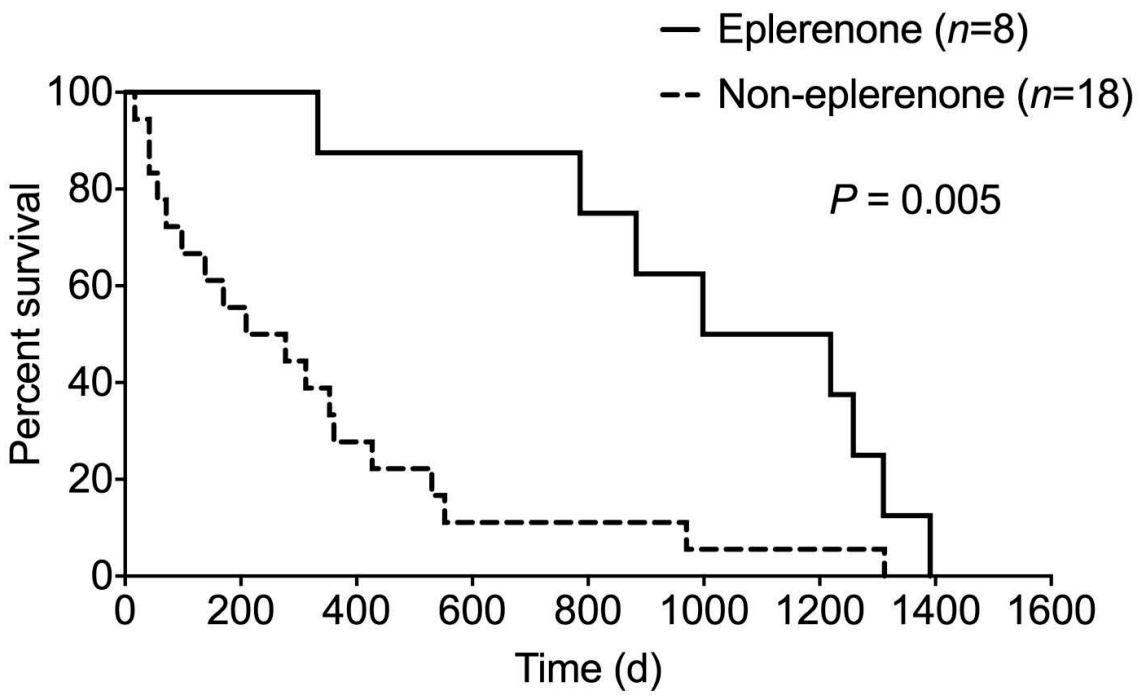

Figure 5. Kaplan–Meier survival curves comparing the survival time of cats treated with eplerenone (solid line) and those that did not receive eplerenone (dashed line) within cats having high plasma aldosterone concentration (PAC) and chronic kidney disease (CKD). *P*-value, showing the statistically significant difference, is presented above the graph.

Discussion

The PAC from 101 healthy, unmedicated cats was investigated in this study to establish normal PAC values. Median PAC was 89 pg/mL, and the upper limit as defined by the 95% percentile was 243 pg/mL. These PACs were very similar to those reported previously (Yu and Morris, 1998; Javadi *et al*., 2004), indicating that the upper limit is appropriate as a reference value in clinically healthy cats. Plasma samples for PAC used in this study were measured collectively from stored plasma within 6 mo. Although it cannot be ruled out that long-term storage would have affected PAC measurements, previous studies in humans have demonstrated the stability of steroid hormones in plasma samples frozen at -25°C for 1 to 10 y (Kley *et al*., 1985).

In the present study, the age in the healthy group was significantly lower than that in the CKD group. This difference is mainly because many blood samples in healthy cats were collected before neutering. There was no significant correlation between age and PAC in this study in healthy cats, consistent with a previous report (Yu and Morris, 1998). Additionally, there was no significant difference in PAC between young, healthy cats and age-matched old healthy cats in this study.

The present study revealed that cats with CKD had significantly higher PACs than healthy cats. Likewise, it has been reported that cats with hypertension and concurrent CKD had increases in PACs and plasma aldosterone:renin ratio (Jensen *et al*., 1997; Jepson *et al*., 2014), suggesting RAAS activation. Additionally, a previous study detected no difference in PAC between normotensive cats with CKD and age-matched controls (Jepson *et al*., 2014). However, in our investigation, PAC was significantly higher in cats with IRIS stage 2 of CKD than healthy

cats. Cats with IRIS Stages 3 and 4 had also significantly higher PAC than healthy cats. In IRIS Stage 4, however, PACs varied greatly among individuals, and some individuals had a low PAC. It is unclear why some cats at the terminal stage of CKD had low PAC. It is reported that cats excrete very small concentrations of free aldosterone and its metabolite, 18-glucuronidated aldosterone, in urine (Syme *et al*., 2007). Polar metabolites of aldosterone interfere with PAC quantification, associated with deteriorating renal function in humans (Jones *et al*., 1981; Koshida *et al*., 1989). Although the influence of polar metabolites on PAC in cats is unknown, it could be important to examine further on aldosterone metabolism and accumulation of polar aldosterone metabolites in feline blood associated with deteriorating renal function. Therefore, when treating with anti-aldosterone drugs based on PAC levels, attention should be given for evaluation of PAC at the terminal stage of CKD in cats.

In cats with CKD, this study revealed that the survival time of cats with high PAC was significantly shorter than that of those with normal PAC. Previously, multiple pathophysiological mechanisms have been proposed to cause shortened survival of cats with high PAC in CKD *via* MR activation, including aldosterone-induced vasculopathy, tubulointerstitial fibrosis, and glomerular injury (Brown, 2013; Spencer *et al*., 2020). Conversely, hypovolemia directly can activate the RAAS and elevates PAC (Papagiannopoulos-Vatopaidinos *et al*., 2020). It is possible that hypovolemia associated with the terminal stages of CKD may be responsible for the short survival in cats with a high PAC.

The MR antagonist, spironolactone, reduces the mortality rate in cats with cardiomyopathy (James *et al*., 2018), but the authors were cautious in concluding this as the cats in the treatment group appeared to have less severe disease than the placebo group. However, the effect of MR antagonist on the mortality rate in cats with CKD has not been fully investigated. Also, the

selective MR antagonist, eplerenone, has not yet been applied to felines. The present study suggests that eplerenone administration significantly prolonged survival of cats with high PAC in CKD. Several of the cats in the prospective study had concurrent cardiac disease and/or arterial hypertension. Positive effects of eplerenone may, at least partially, be due to effects of eplerenone on blood pressure and the heart. Eplerenone reduces hospitalization and mortality in human patients with chronic heart failure, including myocardial infarction (Zannad *et al*., 2011; Ferreira *et al*., 2019). Using MR antagonists to treat end-stage renal disease reduces the 3-y mortality rate and is associated with lower blood pressure, reduced left ventricular mass, and improved left ventricular ejection fraction in human patients (Bomback, 2016). In addition, eplerenone effectively reduced blood pressure compared with other agents, such as spironolactone, enalapril, losartan, and amlodipine in humans (Sierra and Ruilope, 2004). Our results in cats also indicated that although the pathophysiological mechanisms require future studies, eplerenone may reduce mortality rate in cats with high PAC in CKD with cardiovascular disease. Therefore, it is suggested that measuring PAC may be useful, particularly given that eplerenone could be an effective treatment for hyperaldosteronism in cats.

There are some limitations in this study. In the present study, using abdominal ultrasonography, primary hyperaldosteronism caused by adrenal tumors is deemed unlikely in the CKD group. However, as PRA was not measured in most cases of CKD cats, idiopathic hyperplasia of zona glomerulosa tissue might not have been completely excluded. In addition, since the echocardiography was not routinely performed in cats with CKD in this study, asymptomatic heart abnormalities such as HCM may have been overlooked in cats with CKD. Since many cats in this study were receiving medications such as benazepril, furosemide and amlodipine that could potentially affect PAC or stimulate aspects of the RAAS, further analyses

of PAC and survival time in CKD cats under certain conditions with and without medications may be needed. In the eplerenone study, there was no safety data on eplerenone in healthy cats or those with the conditions studied. Although cats were alternately assigned to either the control or eplerenone treatment, they were not randomly assigned eplerenone treatment, and the control group did not receive a placebo treatment. As cats whose owners refused eplerenone treatment were included in the control group, arguably these owners were more cautious or less willing to try treatments, which could have influenced survival. Owners and clinicians were not blinded to the eplerenone treatment. In addition, more than half of the cats in the non-eplerenone treated group had CKD only, compared to only 1/8 in eplerenone-treated group. The higher BUN in some cats of non-eplerenone group may be an indicator that cats were more severely affected in this group, *e.g.*, more dehydrated and/or worse CHF. These findings might have contributed to a difference in survival times between the eplerenone and non-eplerenone groups. Further studies with double-blind, placebo-controlled trials would be required.

In conclusion, cats with CKD had significantly higher PAC than clinically healthy cats. In CKD, the survival of cats with high PAC was significantly shorter than those with normal PAC. The use of eplerenone also significantly prolonged the survival of cats with high PAC in CKD complicated with cardiac disease or hypertension. Although this study proposes PAC as a prognostic marker of CKD cats, eplerenone may be useful in prolonging cats' survival with high PAC in CKD complicated with cardiac disease or hypertension. Our results warrant further studies with double-blind, placebo-controlled trials.

Chapter 2

Plasma aldosterone concentration and the survival of dogs with chronic kidney disease

Introduction

Aldosterone is a steroid hormone synthesized in the adrenal cortex and is part of the renin-angiotensin-aldosterone system (RAAS). It accelerates renal sodium retention and elimination of potassium through its action on the mineralocorticoid receptor (MR) and plays a major role in regulating body fluid volume and blood pressure (Ponda and Hostetter, 2006). The MR is also present in other tissues besides the kidney, including cardiomyocytes and vascular endothelial cells. Aldosterone is locally produced in the vasculature, kidney, and heart in addition to the adrenal gland (Weber *et al.*, 2003), and its actions may induce classical genomic, as well as rapid nongenomic, effects (Brown, 2013). Excessive secretion of aldosterone and activation of the MR cause cardiovascular inflammation, fibrosis and remodeling, and tubulointerstitial fibrosis and glomerular injury in the kidney (Brown, 2013). There are several reports on plasma aldosterone concentration (PAC) in healthy animals, chronic kidney disease (CKD), and chronic heart failure in dogs (Knowlen *et al.,* 1983; Grandt *et al*., 2022). Measurements of urinary aldosterone/creatinine ratio are also reported in dogs (Lantis *et al*., 2015; Galizzi *et al*., 2021). Urinary aldosterone/creatinine ratio is not significantly different between healthy individuals and those with differing stages of myxomatous mitral valve disease in dogs, and is influenced by individual factors such as breed, sex and age (Galizzi *et al*., 2021). It may be important to evaluate PAC in dogs with CKD associated with the activation of RAAS. Although angiotensin-converting enzyme inhibitors and angiotensin receptor blockers are used to suppress the RAAS during renal diseases in dogs (Ames *et al.*, 2019), the relationship between PAC and survival time in dogs with CKD has not been investigated. The purpose of this study was to investigate PAC in dogs with CKD and evaluate the survival time of CKD dogs with high PAC.

Materials and methods

Animals

Records from client-owned dogs that visited the Yasaka Animal Care Center, Japan, between October 2016 and December 2021 that had stored blood samples available were reviewed retrospectively. One hundred and forty-five dogs were identified with the diseases of interest including CKD and systemic hypertension, and categorized into the following groups: healthy (n = 106) and CKD (n = 39). Dogs diagnosed with other concurrent disease were excluded. Informed consent was obtained for the use of the blood samples.

Grouping

The healthy control dogs were animals that visited the hospital for routine health examinations or neutering, had no signs of illness, and received no medication. The dogs were fed a standard commercial diet chosen by each owner and the diet was not specified. Many blood samples in healthy dogs were collected before castration or ovariohysterectomy. Prior to neutering, the dogs were fasted for 8 to 12 hours and allowed to drink water freely. Physical examination, hematological and biochemical examinations including blood urea nitrogen (BUN) and creatinine, and systemic blood pressure measurement were conducted for the health check. Hematological and biochemical examinations, urinalysis, blood pressure measurement, radiography, and abdominal ultrasonography were used to diagnose CKD. Blood chemistries were analyzed using the IDEXX Catalyst One (IDEXX laboratories, Inc., ME). Electrolytes (sodium, potassium and chloride) were measured by ion-selective electrode potentiometry (i-STAT 1 analyzer; Abbott Point of Care, Inc., NJ). Urine protein/creatinine ratio (UPC) or symmetric dimethylarginine (SDMA) level was examined when urinary protein was detected on

dipstick. Dogs with CKD were subsequently classified using the International Renal Interest Society (IRIS) staging of CKD (modified 2019). IRIS stage 1 in this study was defined as: (1) persistent normal plasma creatinine level (<1.4 mg/dL) or normal to mild increase (<18 μg/dL) in SDMA; (2) the presence of any renal abnormality including proteinuria of renal origin, UPC >0.5 persisting for a certain period of time and abnormal renal imaging findings. The study excluded dogs with a temporary and rapid increase in plasma creatinine exceeding the reference value as seen in acute kidney injury. Dogs with kidney diseases including polycystic kidney disease, chronic urinary tract obstruction, chronic urinary tract infection, urolithiasis, Fanconi syndrome were included in the group. In cases such as chronic urinary tract obstruction and urinary tract infection, when the disease was treated and remitted, dogs having chronic renal dysfunction on subsequent follow-up were included in the CKD group. In such diseases, individuals whose CKD-related laboratory values were stable for a certain period of time, approximately one month, were included in the CKD group. Primary hyperaldosteronism, which was diagnosed by abdominal ultrasonography of the adrenal glands, higher PAC and lower renin activity, was not included in this study. Primary hyperaldosteronism due to an adrenocortical tumor was excluded by abdominal ultrasonography. Hypertension was diagnosed through non-invasive measurements using a Doppler (Vet-Dop, Vmed Technology, WA) or oscillometric (Pettrust, Aster Electric Co., Japan) device. Systolic blood pressure (SBP) of ≥160 mmHg was defined as “hypertensive” based on the ACVIM consensus statement (Acierno *et al*., 2018). Blood pressure was measured routinely in all dogs with CKD. It was also routinely measured in healthy dogs for health examination, and if their SBP was non-hypertensive, it was recorded as <160 mmHg in almost of healthy dogs.

Most dogs were receiving no medication at the time of hospital admission, but some dogs had received treatment by the time blood sampling was performed. Seven dogs in the CKD group had received alacepril (1.2–2.4 mg/kg, q12 h or q24 h) or telmisartan (1 mg/kg, q24 h) for 14 days to 3 years.

Sample processing and analysis of PAC

Blood was mixed with ethylenediaminetetraacetic acid and centrifuged. The plasma was then separated and frozen at –35°C until PAC analysis. Plasma samples for PAC measurement were measured collectively for stored plasma within 6 months. The PAC was measured at once in two or more times a year. PAC levels were measured by solid-phase radioimmunoassay, using a kit (SPAC-S Aldosterone Kit, Fujirebio, Tokyo, Japan). The RIA method in the kit used was the same principle as previously validated for use with canine plasma in a commercially available human kit (Hori *et al*., 2008). In this PAC kit, the cross-reactivity with cortisol, corticosterone and progesterone were 0.0002%, 0.03% and 0.008%, respectively, with excellent specificity. Intra- and inter-assay coefficients of variation were 1.8 to 8.3% and 2.4 to 3.2%, respectively. The kit was validated for use in dogs by adding two ranges of aldosterone control extracted from the human matrix to canine plasma. Lower and upper detection limits were determined to be 10 and 1600 pg/mL, respectively, by the standard curve of this kit with excellent sensitivity.

Determination of PAC reference range and survival rates of animals

The reference range for PAC was determined at a 95% confidence interval (CI) by a nonparametric statistic method using values from 106 healthy dogs. With a median PAC of

56 pg/mL, the lower limit was 10 pg/mL at 5% percentile, and the upper limit was 182 pg/mL at 95% percentile. The reference range for normal PAC was defined as 10–182 pg/mL. PAC exceeding the upper limit was defined as "high" PAC. Survival days were calculated from the blood sampling date to compare long-term outcomes between high and normal PAC levels in the CKD group. Five of the 39 CKD dogs were excluded from the survival analysis, because they could not be followed up due to transfer to a different hospital or to another region and their outcome was unknown.

Statistical analysis

Data were analyzed using statistical software (Prism 7.0, GraphPad, CA). Numerical data were tested for normality using the Shapiro–Wilk test. The Mann–Whitney test or unpaired t-test was used to determine the difference between two groups. For multiple group comparison, the nonparametric data were analysed by the Kruskal–Wallis test followed by the post-hoc Dunn's multiple comparison test, and parametric data by one-way analysis of variance and post hoc Tukey's multiple comparison test. Kaplan–Meier curves were constructed to compare survival rates, and log-rank (Mantel-Cox) tests were used to compare survival curves. The significance level for each analysis was at $P < 0.05$. When the numerical data in all groups were abnormally distributed, the data were presented as median and range, whereas normally distributed data were presented as mean ± standard deviation (SD).

Results

Table 6 summarizes the age, sex, breed, blood biochemistry, SBP, and diagnosis of dogs in the healthy and CKD groups as classified by IRIS stage. Age in the healthy group (median 5.9 years) was significantly ($P < 0.01$) younger than that in the CKD group (median 13.8 years). A linear regression analysis showed no significant correlation ($r = 0.008$; $P = 0.94$) between PAC and age in the healthy group (Figure 6). When data in healthy dogs age-matched to CKD group were pulled from the healthy group, there were no significant differences ($P > 0.05$) in PAC, plasma creatinine, and BUN values between young-healthy dogs (< 12 years old; age 5.3 ± 4.1 (mean ± SD), $n = 92$) and old-healthy dogs age-matched to CKD dogs (≥ 12 years old; age 13.7 ± 1.4, $n = 14$). The reason that old-healthy dogs age-matched to CKD dogs were chosen as ≥ 12 years old was because their mean age was most similar to that of the CKD dogs (mean 13.5 years old), with the highest P (= 0.89) value. Five dogs of 106 healthy dogs had a high PAC over the upper limit (>182 pg/mL). The breeds of 5 dogs were 1 Labrador Retriever, 1 Miniature Dachshund, 2 Toy Poodles, and 1 Pug, ranging in age from 6 to 12 years.

Comparison of PAC between healthy and CKD groups

The number of CKD dogs in IRIS stages 1, 2, 3, and 4 were 18, 18, 3, and 0, respectively. Clinical signs in IRIS stage 2 or 3 included emesis, anorexia, anemia, lethargy, polydipsia and polyuria, weight loss, dehydration, or depression. Seven dogs with CKD had hypertension. PAC (median 69 pg/mL; range 10–553 pg/mL) in the CKD group was significantly ($P = 0.03$) higher than that (median 56 pg/mL; range 10–250 pg/mL) in the healthy group (Table 6; Figure 7). The mean ± SD values of PAC in healthy and CKD groups were 73 ± 59 pg/mL and 131 ± 139

pg/mL, respectively. In the CKD group as classified by IRIS stage, PAC (median 97 pg/mL) in IRIS stage 2 and 3 was significantly ($P = 0.03$) higher than in the healthy group (Figure 7). Furthermore, PAC in CKD dogs was also significantly ($P = 0.03$) greater than that (median 43 pg/mL; range 10–177 pg/mL) in age-matched healthy dogs. In the CKD group ($n = 39$), a linear regression analysis showed a significant positive correlation ($r = 0.360$; $P = 0.02$) between PAC and IRIS stage, but no significant correlation ($r = 0.235$; $P = 0.15$) between age and IRIS stage (Figure 8). In the CKD dogs ($n = 34$) used for the survival analysis, a linear regression analysis also showed a significant correlation ($r = 0.351$; $P = 0.03$) between PAC and IRIS stage, but no significant correlation ($r = 0.179$; $P = 0.31$) between age and IRIS stage (Figure 9).

Survival analysis in CKD dogs with high versus normal PAC

Table 7 shows the blood biochemistry, SBP and treatments of dogs with normal and high PAC used for evaluating the survival time in CKD group. Two dogs in the high PAC group had apparent dehydration. Three dogs in the normal PAC group and two dogs in the high PAC group had mild anemia (PCV < 35%). Six dogs in the normal PAC group and five dogs in the high PAC group had mild increase in ALT (> 125 IU/L). Treatments and IRIS stages between normal and high PAC groups were similar (Table 7). On day 0, normal and high PACs (mean ± SD) were 57 ± 36 and 330 ± 121 pg/mL, respectively. In the CKD group, dogs with high PAC had significantly ($P = 0.013$) shorter survival periods than those with normal PAC (Figure 10). Survival time (mean ± SD) of dogs with high PAC and normal PAC was 387 ± 270 days and 742 ± 509 days, respectively. Hazard ratio (high/normal PAC) expressed as 95% CIs for risk of death was 2.35. There were no significant differences in age, body condition score (BCS), plasma creatinine, BUN, plasma potassium, sodium, chloride, inorganic phosphorus, alanine

aminotransferase (ALT), packed cell volume (PCV), total protein, albumin and SBP values between normal and high PAC groups (Table 7). When the cause of death was judged clinically, deaths in the normal PAC group were due to natural or sudden cause in 14 dogs and renal failure in 10 dogs. Deaths in the high PAC group were due to natural or sudden cause in 4 dogs and renal failure in 6 dogs.

Table 6. Age, sex, breed, blood biochemistry, systemic blood pressure and diagnosis of dogs in the healthy and chronic kidney disease (CKD) groups.

Variables	**Healthy**	**CKD**	**IRIS-1**	**IRIS-2 and 3**
Number of dogs	106	39	18	21
Age (y)[a]	5.9 (0.3–17.0)	13.8 (5.2–19.6)**	13.2 (5.2–15.9)**	14.2 (9.4–19.6)**
Male/Female (*n*)	58/48	15/24	5/13	10/11
(Castrated/Ovariohysterectomized)	(36/35)	(5/21)	(1/12)	(4/9)
Breed (*n*)	Toy Poodle (21), Mixed (14), Miniature Dachshund (12), Shiba (8), Chihuahua (5), Pomeranian (4) Labrador Retriever (3), Pembroke Welsh Corgi (3), Bernese Mountain Dog (3), Maltese (3), Shetland Sheepdog (2), Golden Retriever (2), Jack Russell Terrier (2), Pug (2), Shih Tzu (2), Papillon (2), Yorkshire Terrier (2), French Bulldog (2), Poodle (1), Miniature Schnauzer (1), Bulldog (1), American Pit Bull Terrier (1), Border Collie (1), Bichon Frise (1), Samoyed (1), German Shepherd Dog (1), Beagle (1), Chesapeake Bay Retriever (1), Doberman Pinscher (1), Cavalier King Charles Spaniel (1), Miniature Pinscher (1), Italian Greyhound (1)	Miniature Dachshund (7), Labrador Retriever (4), Toy Poodle (4), Pembroke Welsh Corgi (4), Mixed (3), Shiba (3), Miniature Schnauzer (3), Border Collie (2), Yorkshire Terrier (2), Shetland Sheepdog (1), Poodle (1), Golden Retriever (1), Maltese (1), Chihuahua (1), Hokkaido dog (1), Weimaraner (1)	Labrador Retriever (3), Toy Poodle (2), Pembroke Welsh Corgi (2), Yorkshire Terrier (2), Mixed (1), Shiba (1), Miniature Schnauzer (1), Border Collie (1), Shetland Sheepdog (1), Maltese (1), Chihuahua (1), Hokkaido dog (1), Weimaraner (1)	Miniature Dachshund (7), Toy Poodle (2), Pembroke Welsh Corgi (2), Mixed (2), Shiba (2), Miniature Schnauzer (2), Labrador Retriever (1), Border Collie (1), Poodle (1), Golden Retriever (1)
Diagnosis (*n*)	None (106)	IRIS-1 (18) IRIS-2 (18) IRIS-3 (3)	IRIS-1 (18)	IRIS-2 (18) IRIS-3 (3)

Cause of CKD (*n*)		Glomerulonephritis (8), Urolithiasis (5), Polycystic kidney disease (5), Fanconi syndrome (3), Chronic urinary tract infection (2), Pyelonephritis (1), Atrophy of left kidney (1), Unknown including aging (14)	Glomerulonephritis (6), Fanconi syndrome (2), Chronic urinary tract infection (2), Unknown including aging (8)	Urolithiasis (5), Polycystic kidney disease (5), Glomerulonephritis (2), Fanconi syndrome (1), Pyelonephritis (1), Atrophic kidney (1), Unknown including aging (6)
Medication (*n*)		Alacepril (1.2–2.4 mg/kg, q12 h or q24 h) for 14 days to 3 years (6), Telmisartan (1 mg/kg, q24 h) for 90 days (1), Antibiotics (1)	Alacepril (1.4–2.4 mg/kg, q12 h or q24 h) for 30 days to 7 months (3), Telmisartan (1 mg/kg, q24 h) for 90 days (1), Antibiotics (1)	Alacepril (1.2–2 mg/kg, q12 h or q24 h) for 14 days to 3 years (3)
Blood urea nitrogen (mg/dL)[a]	18 (6–40)	23 (10–188)**	22 (11–52)*	30 (10–188)**
Plasma creatinine (mg/dL)[a]	0.9 (0.4–1.4)	1.4 (0.7–4.3)**	1.2 (0.7–1.4)	1.8 (1.4–4.3)**[†]
Systolic blood pressure (mmHg)[b]	<160	147 ± 15	150 ± 16	143 ± 13
Plasma aldosterone concentration (PAC) (pg/mL)				
Median (range)[a]	56 (10–250)	69 (10–553)*	58 (10–355)	97 (10–553)*
Mean ± SD[b]	73 ± 59	131 ± 139	99 ± 97	159 ± 165

IRIS – International Renal Interest Society.

[a] Median (minimum–maximum).

[b] Mean ± standard deviation.

* $P < 0.05$, ** $P < 0.01$, significantly different from healthy group.

[†] $P < 0.01$, significantly different from IRIS-1 group.

Table 7. Blood biochemistry, systemic blood pressure and medications of dogs with normal and high PAC used for survival analysis in chronic kidney disease (CKD) group.

Variables	**Normal PAC**	**High PAC**
Number of dogs	24	10
Age (y)[a]	13.4 ± 2.2	14.6 ± 1.7
Male/Female (*n*) (Castrated/Ovariohysterectomized)	10/14 (2/12)	3/7 (3/6)
Body condition score (five-point system)[b]	3 (2.5–4)	3 (2.5–4)
IRIS stage (% incidence)	IRIS-1 (42%) IRIS-2 (54%) IRIS-3 (4%)	IRIS-1 (30%) IRIS-2 (50%) IRIS-3 (20%)
Cause of CKD (*n*)	Glomerulonephritis (7), Urolithiasis (4), Fanconi syndrome (3), Polycystic kidney disease (2), Chronic urinary tract infection (1), Pyelonephritis (1), Atrophy of left kidney (1), Unknown including aging (5)	Polycystic kidney disease (3), Glomerulonephritis (1), Urolithiasis (1), Chronic urinary tract infection (1), Unknown including aging (4)
Duration of disease (months)[a]	12.1 ± 8.7	10.5 ± 6.8
Plasma aldosterone concentration (PAC) (pg/mL)[a]	57 ± 36	330 ± 121**
Blood urea nitrogen (mg/dL)[b]	28 (10–88)	26 (11–188)
Plasma creatinine (mg/dL)[b]	1.6 (0.7–4.1)	1.5 (1.0–4.3)
Plasma potassium (mmol/L)[a]	4.6 ± 0.4	4.7 ± 0.8
Plasma sodium (mmol/L)[a]	146 ± 3	144 ± 5
Plasma chloride (mmol/L)[a]	114 ± 4	115 ± 3
Plasma inorganic phosphorus (mg/dL)[a]	4.4 ± 1.1	5.7 ± 4.1
Plasma alanine aminotransferase (IU/L)[a]	111 ± 106	162 ± 130
Packed cell volume (%)	43.4 ± 6.0	43.2 ± 7.3
Total protein (g/dL)	6.9 ± 0.6	6.7 ± 0.5
Systolic blood pressure (mmHg)[a]	147 ± 18	148 ± 11

Treatment (*n*)	Fluid infusion (7), Alacepril 1.2-2.4 mg/kg, SID or BID (6), Telmisartan 1 mg/kg, SID (3), Phosphate binder (3), Kidney support diet[c] (2), or Antibiotics (1)	Fluid infusion (4), Alacepril 1.4-2.0 mg/kg, SID or BID (5), Phosphate binder (1), or Antibiotics (1)
Survival time (day)[a]	742 ± 509	387 ± 270*

PAC – Plasma aldosterone concentration.

IRIS – International Renal Interest Society.

[a] Mean ± standard deviation.

[b] Median (minimum–maximum).

[c] Specific diet formulated with low phosphorous and restricted or highly digestible protein of high quality (Royal Canin; Canine Renal Support A Dry Dog Food or Canine Multifunction Renal Support + Hydrolyzed Protein Dry Dog Food).

* $P < 0.05$, ** $P < 0.01$, significantly different from normal PAC.

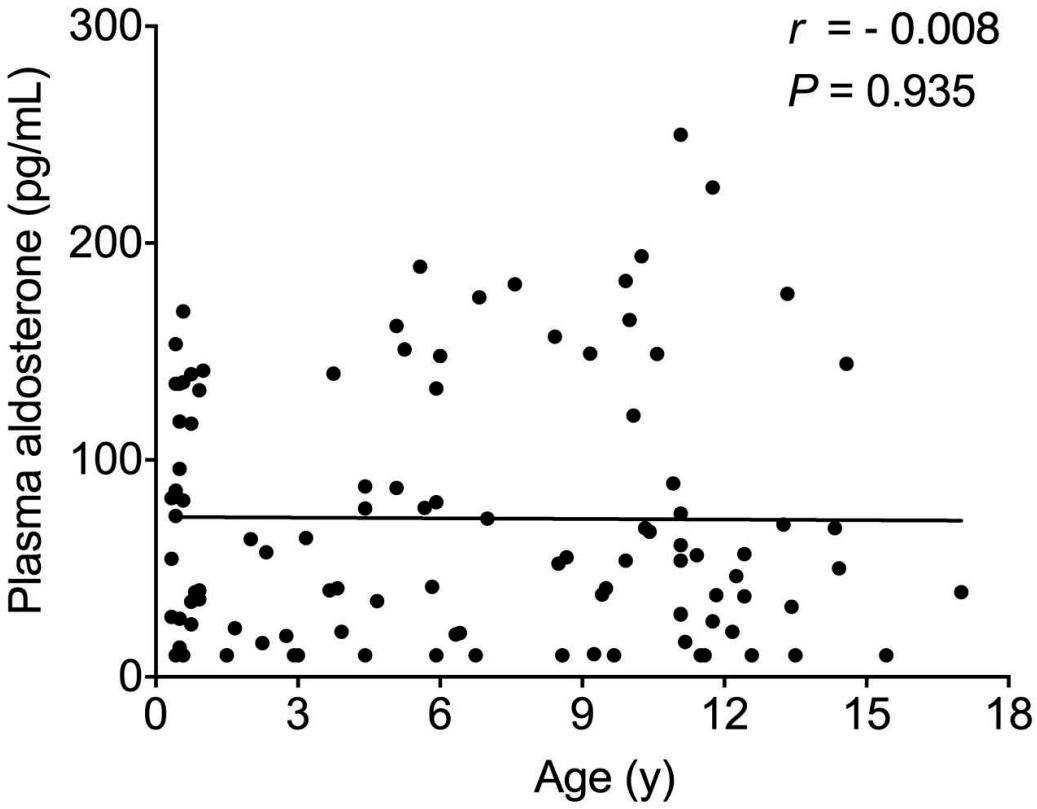

Figure 6. Correlation between plasma aldosterone concentration and age in 106 healthy dogs. Data were analysed using linear regression analysis. A correlation coefficient (*r*) and *P*-value are presented above the graph.

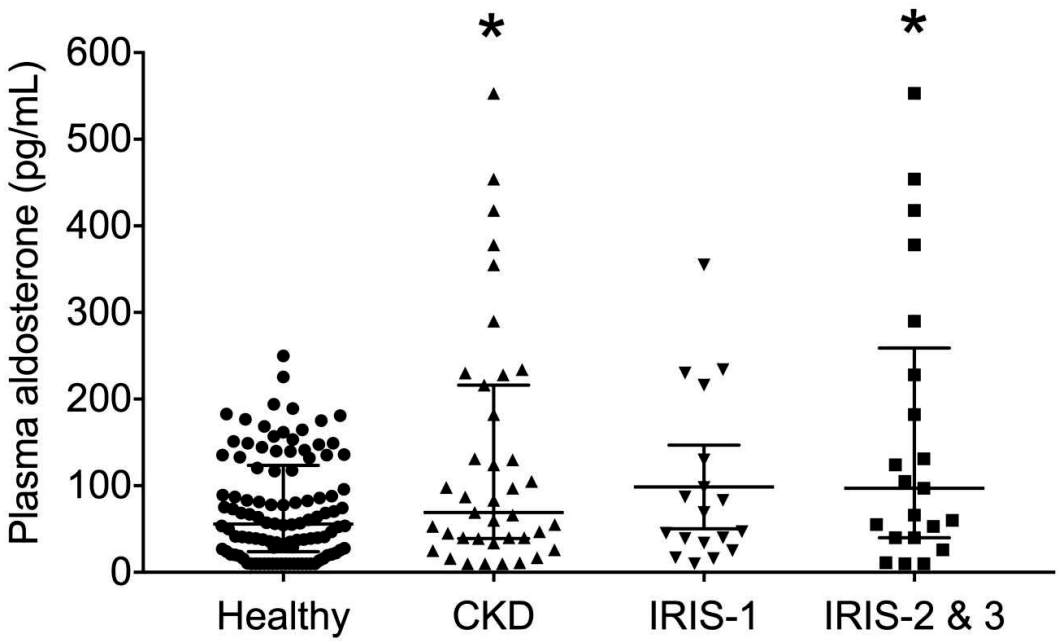

Figure 7. Plasma aldosterone concentration in healthy (n = 106) and chronic kidney disease (CKD) (n = 39) groups, and groups as classified by IRIS stage 1 (n = 18), and IRIS stage 2 and 3 (n = 21). The bars and whiskers indicate the median, 25th, and 75th quartiles. * $P < 0.05$, showed significant difference from the healthy group.

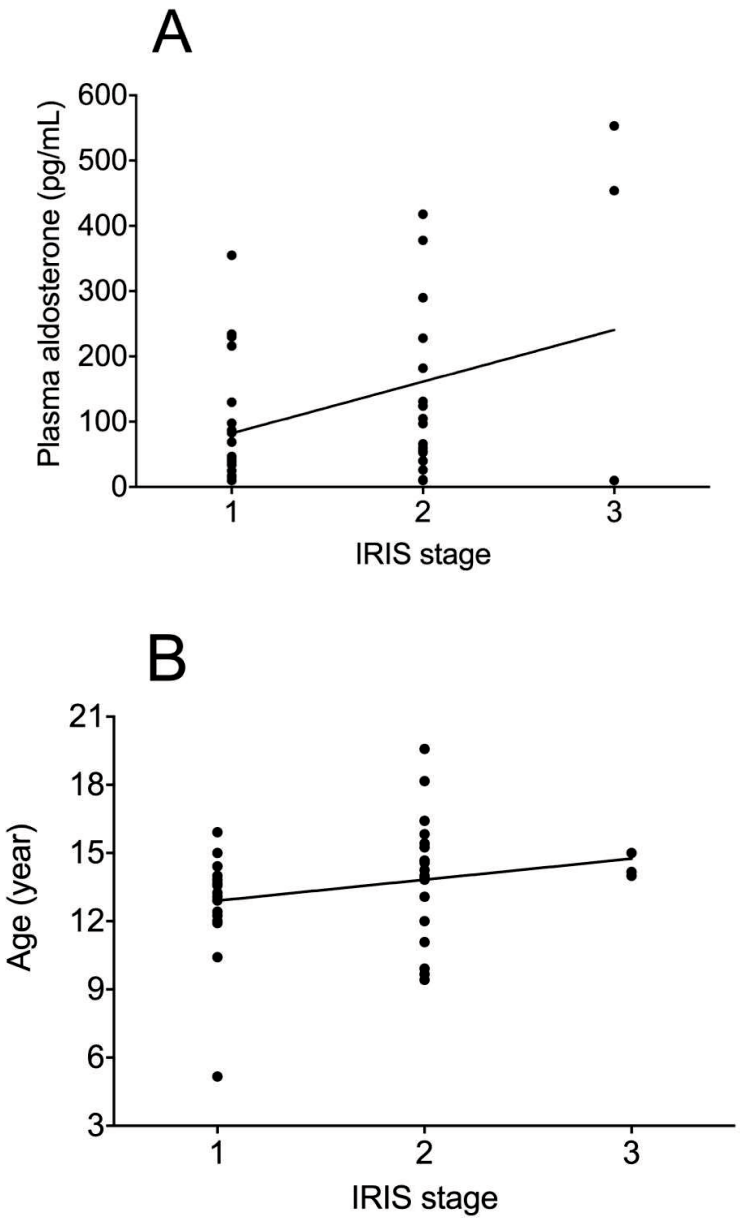

Figure 8. Correlations between IRIS stage and plasma aldosterone concentration (A) and between IRIS stage and age (B) in the CKD group (n = 39). A linear regression analysis showed a significant positive correlation (r = 0.360; P = 0.02) between PAC and IRIS stage, but no significant correlation (r = 0.235; P = 0.15) between age and IRIS stage.

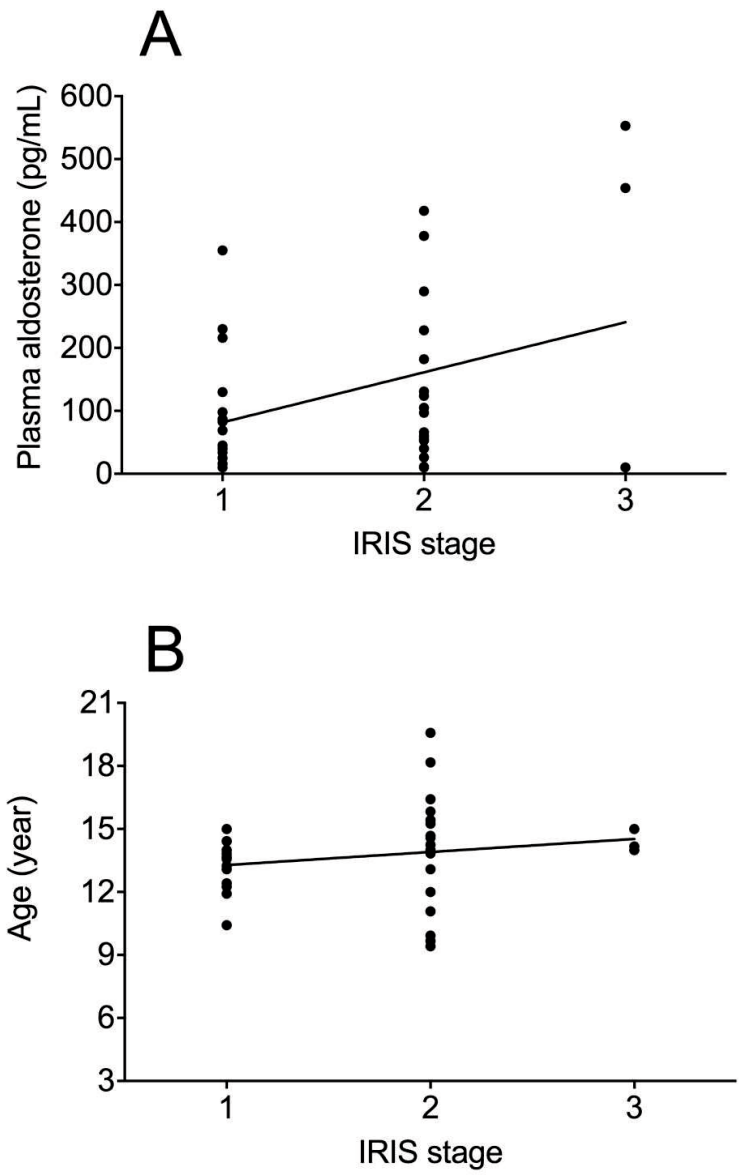

Figure 9. Correlations between IRIS stage and plasma aldosterone concentration (A) and between IRIS stage and age (B) in the CKD dogs (n = 34) used for the survival analysis. A linear regression analysis showed a significant correlation (r = 0.351; P = 0.03) between plasma aldosterone concentration and IRIS stage, but no significant correlation (r = 0.179; P = 0.31) between age and IRIS stage.

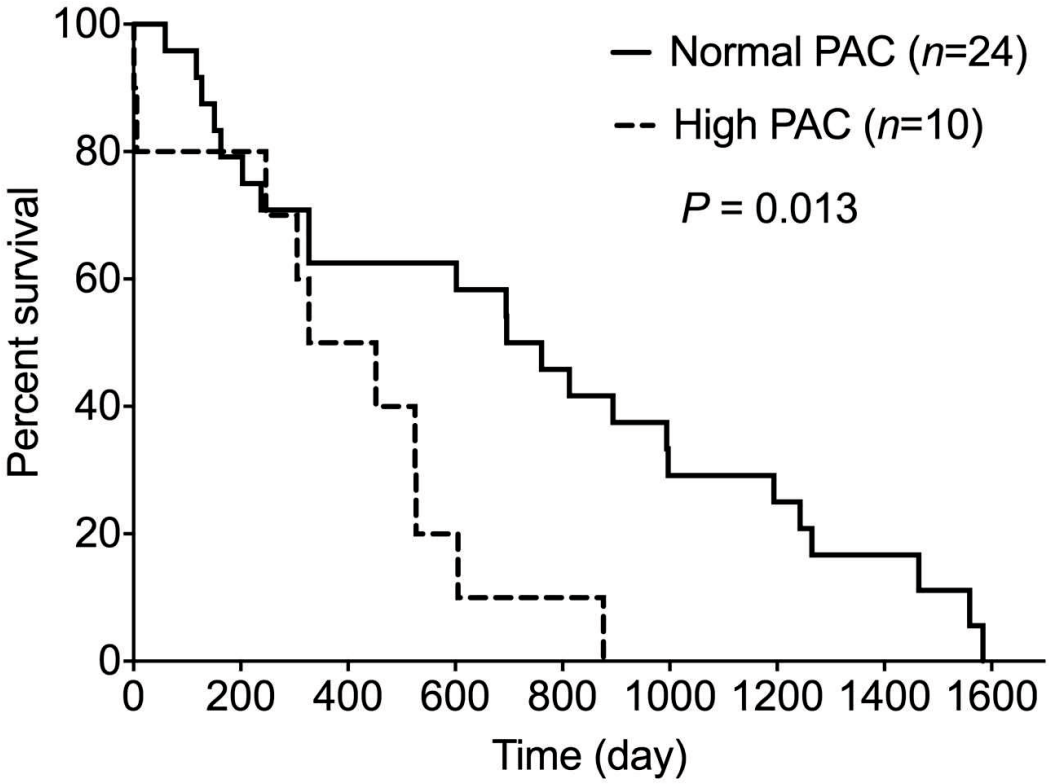

Figure 10. Kaplan–Meier survival curves comparing survival time of dogs with normal (solid line) and high (dashed line) plasma aldosterone concentration (PAC) in the chronic kidney disease (CKD) group. While high PAC was set at > 182 pg/mL, normal PAC was 10–182 pg/mL. *P*-value, showing statistically significant differences, is presented above the graph.

Discussion

The PAC from 106 healthy, unmedicated dogs was investigated in this study to establish normal PAC values. Results showed that the median PAC was 56 pg/mL, and the upper limit as defined by the 95% percentile was 182 pg/mL. To the best of our knowledge, there are no published reports on the reference value of PAC using more than 100 healthy dogs. However, median PAC in this study was similar to those (mean 43–76 pg/mL, n = 12–24) on healthy dogs reported previously (Knowlen *et al.*, 1983; Javadi *et al.*, 2003). Furthermore, the upper limit of PAC in the present study was similar to the maximum value (143–190 pg/mL) in small populations of healthy dogs (n = 10–24) in previous studies (Knowlen *et al.*, 1983; Baumstark *et al.*, 2014; Gójska-Zygner and Zygner, 2015), suggesting that the upper limit is appropriate as a reference value in healthy dogs. The upper limit of PAC in healthy dogs of this study was also similar to that (195 pg/mL) in a large population of healthy cats (n = 130) reported previously (Javadi *et al.*, 2004). On the other hand, five dogs of 106 healthy dogs had a high PAC over the upper limit in this study. The PAC in dogs is influenced by individual factors such as breed, sex and age (Pedersen *et al.*, 1995; Galizzi *et al.*, 2021). Although breed differences in PAC levels of healthy dogs have been reported (Pedersen *et al.*, 1995), the five dogs are not always included in the breed having a high PAC in that report. Furthermore, from history taking and physical examination, these dogs showed no evidence of dietary management or hydration status problems. However, four out of these five dogs were over 10 years of age, thus subclinical adrenal or renal abnormalities that could not be diagnosed by physical examination, renal panel, and imaging should not be completely ignore. The PACs in the present study were measured collectively from stored plasma within 6 months. Although it cannot be ruled out that long-term

storage would have affected PAC measurements, previous studies in humans have demonstrated the stability of steroid hormones in plasma samples frozen at -25°C for 1 to 10 years (Kley *et al.*, 1985). Therefore, the effect of different storage time might be minimal in the present study.

In the present study, age in the healthy group was significantly younger than that in the CKD group. This difference is mainly because many blood samples of the healthy dogs were from young healthy dogs referred for health screening before neutering. However, linear regression analysis showed no significant correlation between PAC and age in the healthy group. This result in healthy dogs was consistent with a previous report in cats (Yu and Morris, 1998). Additionally, when data in healthy dogs age-matched to CKD group were pulled from the healthy group, there was no significant difference in PAC between young, healthy dogs and age-matched old, healthy dogs in this study.

The present study revealed that dogs with CKD, especially classified by IRIS stage 2 and 3, had significantly higher PACs than healthy dogs. PACs in dogs with IRIS stage 2 and 3 of this study were similar to those (median 83 pg/mL, $n = 10$) in canine CKD reported recently (Grandt *et al.*, 2022). Likewise, it has been reported that cats with hypertension and concurrent CKD had increases in PACs and plasma aldosterone/renin ratio (Jensen et al., 1997; Jepson et al., 2014), suggesting RAAS activation.

In dogs with CKD, this study revealed that the survival time of dogs with high PAC was significantly shorter than that of those with normal PAC. Previously, multiple pathophysiological mechanisms have been proposed to cause shortened survival of cats with high PAC in CKD via MR activation, including aldosterone-induced vasculopathy, tubulointerstitial fibrosis, and glomerular injury (Brown, 2013; Spencer *et al.*, 2020). On the other hand, hypovolemia directly can activate the RAAS and elevates PAC (Papagiannopoulos-Vatopaidinos *et al.*, 2020). It is

possible that hypovolemia associated with the higher stages of CKD may be responsible for the short survival in dogs with a high PAC. Therefore, it is suggested that measuring PAC may be useful for a prognostic marker of dogs with CKD. The MR antagonists may prolong the survival of CKD dogs with high PAC, although future investigations are required. Additionally, in the present study, a significant positive correlation between PAC and IRIS stage was observed in CKD dogs used for survival analysis, suggesting that the lower survival rate in high PAC group may be related to severity of CKD as well as effect of aldosterone.

Diet effect on the PAC level was also reported in previous dog studies. For example, potassium-depleted diet and high sodium intake decreased PAC (Hulter *et al.*, 1980; Kjolby *et al.*, 2005), while sodium-depletion diet elevated PAC in dogs (Lovern *et al.*, 2001; Bie *et al.*, 2009). In the present study, feeding for the dogs was not a sodium or potassium restricted diet. In addition, there were no significant differences in breed, sex, age and plasma sodium, potassium and chloride levels between the high PAC and normal PAC groups, suggesting that these factors may not largely influence the PAC level in CKD dogs of this study. On the other hand, PAC is reported to be increased by anemia (Anand *et al.*, 1993), obesity (Dinh Cat *et al.*, 2016), azotemic hypertention (Jepson *et al.*, 2014) and chronic liver disease (El-Raziky *et al.*, 2005). Chronic liver dysfunction may reflect the inability of the diseased liver to metabolize and clear plasma aldosterone from the circulation (El-Raziky *et al.*, 2005). In the present study, some of CKD dogs in both high PAC and normal PAC groups had azotemia, mild anemia, mild increase in hepatic enzyme (ALT) and increase in BCS, which may have influenced the PAC value. However, there were no significant differences in blood biochemistry and BCS between the high and normal PAC groups in CKD dogs of this study. Additionally, age of dog and duration of CKD as well as other complications may affect the survival time in CKD dogs. In the present

study, the age of dog and duration of CKD at the time of blood sampling for PAC measurement did not significantly differ between the high and normal PAC groups, suggesting that these factors may not have had a significant effect on the difference in survival time between the two groups.

There are some limitations in this study. In the present study, using abdominal ultrasonography, primary hyperaldosteronism caused by adrenal tumors is deemed unlikely in the CKD group. However, as plasma renin activity was not measured in most cases of CKD dogs, idiopathic hyperplasia of zona glomerulosa might not have been completely excluded. It has been mentioned that PAC value should be measured after the discontinuation of medications affecting this value; the duration of discontinuation is recommended >2 weeks for angiotensin converting enzyme inhibitors and angiotensin II receptor antagonists (Tamura *et al.*, 2021). In the present study, some dogs in the CKD group had received these medications at the time of blood sampling for PAC evaluation. In addition, since many dogs used for survival analysis in this study were receiving medications such as alacepril and telmisartan that could potentially affect PAC or stimulate aspects of the RAAS, further analyses of PAC and survival time in a large population of CKD dogs under certain conditions with and without medications should be performed. Small sample size and uneven number of dogs between groups in this study might also be included a problem for statistical power.

In conclusion, dogs with CKD had significantly higher PAC than healthy dogs. In CKD, the survival of dogs with high PAC was significantly shorter than those with normal PAC. A significant positive correlation between PAC and IRIS stage was observed in CKD dogs, suggesting that the lower survival rate in high PAC group may be related to severity of CKD. Therefore, high PAC might indicate shorter survival time in dogs with CKD. However, further

study on PAC level in CKD progression and treatment response in a larger population should be performed.

General Conclusion

In chapter 1, the study aimed to investigate the plasma aldosterone concentration (PAC) in cats with chronic kidney disease (CKD), and retrospectively evaluate the survival of cats with high PAC. Furthermore, this study prospectively examined eplerenone's effect on survival time in CKD cats with high PAC. The eplerenone study was conducted including both cats with CKD only and CKD cats complicated cardiac disease or systemic hypertension. The PAC was measured retrospectively in blood samples obtained from 156 client-owned cats that visited a veterinary hospital. The cats were designated into 2 groups: clinically healthy (n = 101) and CKD (n = 55). The PAC was measured by solid-phase radioimmunoassay; median (minimum–maximum) PAC in healthy cats was 97 (10–416) pg/mL and the upper limit (95th percentile) was 243 pg/mL. In the CKD group, PAC [126 (10–981) pg/mL] was higher ($P < 0.01$) than in the clinically healthy group. In the CKD group as classified by IRIS stage, the PACs were higher ($P = 0.03$) in IRIS stage 2 than in the healthy group. Similarly, PACs in IRIS stage 3 and 4 cats were higher ($P = 0.01$) than in the healthy group. In cats with CKD, the survival time of those with high PAC (n = 16) (> 243 pg/mL) was shorter ($P = 0.019$) than that of those (n = 39) with normal PAC. In cats with high PAC and CKD, administering an aldosterone antagonist, eplerenone, at 2.5 to 5 mg/kg body weight (n = 8) prolonged the survival ($P = 0.005$), compared to cats not receiving eplerenone (n = 18). In conclusion, the present study revealed that cats with CKD had a high PAC. The PAC could be a prognostic marker of CKD in cats. Eplerenone may prolong the survival in cats with CKD and a high PAC complicated with cardiac disease or hypertension.

In chapter 2, the study aimed to investigate PAC in dogs with CKD and evaluated the survival of CKD dogs with high PAC, retrospectively. PAC was measured in blood samples obtained from 145 client-owned dogs. The dogs were divided into two groups: healthy (n = 106) and CKD (n = 39). In healthy group, median (minimum–maximum) PAC was 56 (10–250) pg/mL, and the upper limit (95 percentile) was 182 pg/mL. PAC (median 69 pg/mL; range 10–553 pg/mL) in CKD group was significantly ($P < 0.05$) higher than in the healthy group. In the CKD group as classified by IRIS stage, PAC (median 97 pg/mL) in IRIS stage 2 and 3 was significantly higher than in the healthy group. A significant positive correlation between PAC and IRIS stage was observed in CKD dogs, suggesting that the lower survival rate in high PAC group may be related to severity of CKD. In dogs with CKD, the survival time (mean ± SD; 387 ± 270 days) of those with high PAC (n = 10) (> 182 pg/mL) was significantly ($P < 0.05$) shorter than that (742 ± 509 days) of those (n = 24) with normal PAC. In conclusion, the present study revealed that dogs with CKD had a high PAC. The high PAC might indicate shorter survival time in dogs with CKD. However, further study on PAC level in CKD progression and treatment response in a larger population may be needed.

Abstract

Aldosterone is a steroid hormone synthesized in the adrenal cortex and is part of the renin-angiotensin-aldosterone system (RAAS). It accelerates renal sodium retention and elimination of potassium through its action on the mineralocorticoid receptor (MR), and has a major role in regulating body fluid volume and blood pressure. Excessive secretion of aldosterone and activation of the MR cause cardiovascular inflammation, fibrosis and remodeling, and tubulointerstitial fibrosis and glomerular injury in the kidney. There are several reports on plasma aldosterone concentration (PAC) in healthy, chronic kidney disease (CKD), systemic hypertension, and chronic heart failure in cats and dogs. Measurement of urinary aldosterone/creatinine ratio has also been reported in cats and dogs. However, it has been suggested that measuring aldosterone in feline urine using the available methodology has limited or no utility in investigating feline hypertension associated with CKD. It may be important to evaluate PAC in cats and dogs with CKD associated with the activation of RAAS.

On the other hand, angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers suppress the RAAS during hypertensive, renal, and cardiac diseases in cats. The Randomized Aldactone Evaluation Study in humans showed that the aldosterone antagonist, spironolactone, reduced the mortality of patients with chronic heart failure who received ACEI and loop diuretics. Spironolactone also reportedly reduced the mortality rate in cats with congestive heart failure secondary to cardiomyopathy. Another selective aldosterone antagonist, eplerenone, not only antagonizes MR but also blocks the nongenomic effects of aldosterone in vascular tissues not susceptible to spironolactone. These effects of eplerenone may be more effective than spironolactone in treating hypertension due to vasoconstriction. Although

eplerenone reduces mortality and hospitalization in human patients with chronic heart failure, there are no available reports on eplerenone's use in feline practice.

Since elevated PAC is a risk factor for kidney injury in humans, and MR antagonists are beneficial in rodent models of CKD and human patients, it was hypothesized that if an elevated PAC is detectable in the early stages of the disease in cats, the use of eplerenone may prolong lifespan. However, the relationship between PAC and the survival time in cats and dogs with CKD has not been investigated. Therefore, this study aimed to investigate PAC in cats and dogs with CKD, and evaluate the influence of high PAC on the survival time of CKD animals and the effect of treatment with eplerenone in CKD cats with high PAC.

In chapter 1, PAC in cats with CKD was investigated retrospectively, and the survival time of cats with high PAC was evaluated. Furthermore, the effect of treatment with eplerenone on survival time in CKD cats with high PAC was examined prospectively. The eplerenone study was conducted including both cats with CKD only and CKD cats complicated cardiac disease or systemic hypertension. The PAC was measured retrospectively in blood samples obtained from 156 client-owned cats that visited a veterinary hospital. The cats were designated into 2 groups: clinically healthy (n = 101) and CKD (n = 55). The PAC was measured by solid-phase radioimmunoassay. Median (minimum–maximum) PAC in healthy cats was 97 (10–416) pg/mL and the upper limit (95th percentile) was 243 pg/mL. In the CKD group, PAC [126 (10–981) pg/mL] was significantly higher than in the clinically healthy group. In the CKD group as classified by the International Renal Interest Society (IRIS) stage, the PACs were higher in IRIS stage 2 than in the healthy group. Similarly, PACs in IRIS stage 3 and 4 cats were higher than in the healthy group. In cats with CKD, the survival time of those with high PAC (n = 16) (> 243 pg/mL) was significantly shorter than that of those (n = 39) with normal PAC. In cats with

high PAC and CKD, eplerenone administration (2.5 to 5 mg/kg body weight; *n* = 8) prolonged significantly the survival compared to cats not receiving eplerenone (*n* = 18). These results indicated that PAC could be a prognostic marker of CKD in cats and that eplerenone may prolong the survival in cats with CKD and high PAC complicated with cardiac disease or hypertension.

In chapter 2, PAC in dogs with CKD was investigated retrospectively, and the survival time of CKD dogs with high PAC was evaluated. PAC was measured in blood samples obtained from 145 client-owned dogs. The dogs were divided into two groups: clinically healthy (*n* = 106) and CKD (*n* = 39). In clinically healthy group, median (minimum–maximum) PAC was 56 (10–250) pg/mL, and the upper limit (95th percentile) was 182 pg/mL. PAC (median 69 pg/mL; range 10–553 pg/mL) in CKD group was significantly higher than in the healthy group. In the CKD group as classified by IRIS stage, PAC (median 97 pg/mL) in IRIS stage 2 and 3 was significantly higher than in the healthy group. A significant positive correlation between PAC and IRIS stage was observed in CKD dogs, suggesting that the lower survival rate in high PAC group may be related to severity of CKD. In dogs with CKD, the survival time of those with high PAC (*n* = 10) (> 182 pg/mL) was significantly shorter than that of those with normal PAC (*n* = 24). These results suggested that high PAC might indicate shorter survival time in dogs with CKD.

In conclusion, this study revealed that both cats and dogs with CKD had significantly higher PAC than clinically healthy animals. In CKD, the survival time of cats and dogs with high PAC was significantly shorter than those with normal PAC. The use of eplerenone also significantly prolonged the survival of cats with high PAC in CKD complicated with cardiac disease or hypertension. This study proposes PAC as a prognostic marker of cats or dogs with CKD. Eplerenone may be useful in prolonging cats' survival with high PAC in CKD complicated with

cardiac disease or hypertension. However, further study on PAC level in CKD progression and treatment response in a larger population may be required. This study provided new information on the relationship between PAC and the survival of cats or dogs with CKD, and the effect of eplerenone treatment for the survival time of cats with high PAC and CKD.

Acknowledgements

I would like to thank my doctoral course supervisor Professor Dr. Yoshiaki Hikasa (Tottori University) for his guidance and support throughout my graduate student years, and for the critical reviewing of the manuscript. He is a talented and brilliant scientist and a great mentor, from whom I have learned a great deal.

I am grateful to Professor Dr. Akira Yabuki (Kagoshima University) for their guidance as assistant supervisors during my study. Special thanks to Professor Dr. Tomohiro Imagawa (Tottori University), Professor Dr. Takashi Takeuchi (Tottori University), and Associate Professor Dr. Kazuki Harada (Tottori University) for the review of this doctoral thesis.

I am also grateful to Drs. Sumie Terunuma and Noriaki Ihara for their assistance with data collection. I also wish to thank the dog's owners for their involvement in the study.

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