Communication

Renin-Angiotensin Aldosterone System Modulation and Cardiovascular-Kidney-Metabolic Health

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Renin-Angiotensin Aldosterone System Modulation and Cardiovascular-Kidney-Metabolic Health

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Abstract: Similar to humans, the renin-angiotensin-aldosterone system (RAAS) plays a key role in regulating blood pressure and body fluid homeostasis in dogs and cats. The classical RAAS pathway, which involves the conversion of angiotensin I to angiotensin II by the angiotensin converting enzyme (ACE), leads to aldosterone production and associated maladaptive effects in cardiovascular and renal diseases. Recent studies have highlighted the alternative RAAS pathway, which counteracts these effects through angiotensin 1-7, promoting vasodilation and reducing inflammation. Beyond RAAS modulation, RAAS inhibitors have been shown to exert significant anti-inflammatory properties. This is particularly relevant given the American Heart Association's recent focus on Cardiovascular-Kidney-Metabolic (CKM) health, which emphasizes the interconnected risks of cardiovascular disease, chronic kidney disease (CKD), type 2 diabetes, and obesity. These conditions share common pathophysiological pathways, including chronic inflammation, oxidative stress, and metabolic dysregulation. Advances in biomedical research, including the development of adult stem cell-derived canine organoids, provide impetus to study the effects of RAAS modulators and new CVD drugs on CKM health, with a focus on epithelial inflammation within the context of One Health.

Keywords: RAAS; Cardiorenal Metabolic Diseases; Inflammation; One Health

What we know about the renin-angiotensin aldosterone system in dogs and cats

The renin-angiotensin-aldosterone system (RAAS is integral to regulating blood pressure (BP) and fluid balance (Hall et al., 1989). The classical RAAS pathway refers to the peptide cascade involving the conversion of angiotensin I (ANG I) to angiotensin II (ANG II) by angiotensin-converting enzyme (ACE), eventually leading to increased adrenal production of aldosterone (ALD). Physiological consequences of ANG II and ALD include vasoconstriction, sodium and water retention, and myocardial and vascular fibrosis, which are considered maladaptive in the context of cardiovascular and renal diseases (Young et al., 1994; Shiffrin, 2006; Waanders et al., 2011). In addition to the ANG II-ALD axis, it is now well-recognized that the RAAS also includes additional signaling pathways that balance the effects of the classical RAAS. Specifically, the alternative RAAS pathway involves the conversion of ANG II by the enzyme angiotensin-converting enzyme 2 (ACE2) into angiotensin 1-7 (Ang 1-7), with downstream signaling leading to vasodilation, diuresis, natriuresis, and mitigation of vascular inflammation (Esteban et al., 2009). Therefore, the alternative RAAS pathway provides an internal counterregulatory mechanism that can partly mitigate the negative effects of ANG II and ALD. In theory, the ideal RAAS-modulating therapy would downregulate the classical RAAS and upregulate the alternative RAAS pathway (Arendse et al., 2019).

There is scarce information about the activation of the RAAS in dogs with congestive heart failure (CHF), independent of treatment. However, multiple clinical trials have demonstrated a clinical benefit of RAAS modulators, such as ACE inhibitors (The COVE Study Group, 1995; The IMPROVE Study Group, 1995; BENCH, 1999; Besche et al., 2007), or mineralocorticoid receptor antagonists (MRA) (Coffman et al., 2021) in dogs with cardiac diseases. A recent retrospective study by Ward et al. (2021) also showed that administering a higher dose of an ACE inhibitor was linked

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to improved survival rates in dogs with initial onset of CHF. Furthermore, among the canine subgroup with CHF during the ACE inhibitor prescription, a higher dose was linked to better chances of survival at two years. In addition, the physiology of the RAAS and its response to RAAS inhibition has been extensively studied in experimental models of RAAS activation by our group (Mochel et al., 2013a, b; 2014, 2015, 2019; Mochel & Danhof, 2015; Sotillo et al., 2023; Schneider et al., 2023).

In cats with systemic hypertension (SHT), the activation of RAAS components can vary:

- **Plasma renin activity (PRA)**. Jepson et al. (2014) reported low PRA levels in cats with SHT, while a previous retrospective study from our consortium found elevated PRA levels in SHT cats given amlodipine, but no differences in PRA in untreated SHT patients compared to healthy controls (Ward et al., 2022).
- **Angiotensins I and II**. Similarly, our 2022 retrospective study found a significant elevation in ANG I (but not ANG II) levels in cats with SHT who were treated with amlodipine, but no differences were found in untreated SHT patients compared to healthy controls.
- **Aldosterone**. Jepson et al. (2014) reported elevated ALD levels in cats with SHT. Consistent with our findings on PRA and ANG I, elevation in ALD was only observed in SHT cats treated with amlodipine in our 2022 retrospective study.

These changes in RAAS due to amlodipine were later confirmed in a prospective study, which included 20 healthy cats treated with AML besylate (0.625 mg PO q24h) for 14 days. The study showed increases in ANG I, ANG II, and PRA compared to the placebo, but no significant changes were seen in ALD (Garcia Marrero et al., 2024). Both studies (Ward et al., 2022; Garcia Marrero et al., 2024) also reported an increase in Ang 1-7, which could potentially be associated with positive clinical outcomes in feline patients with SHT.

In cats with cardiomyopathy, data from our retrospective study (Ward et al., 2022) showed consistent increases in PRA, ANG I and ALD compared with healthy cats, and these differences remained significant after considering subgroups of untreated or furosemide-treated cats.

In cats with chronic kidney disease (CKD), a previous study by Mishina et al. (1998) reported a significant elevation in ANG II levels in seven cats with renal disease compared to the control group (N=11). However, the study had several limitations, including a lack of methodological information and a small sample size. Additionally, the study lacked information on the cats systolic BP, and a clear definition for the “normal” (control) group.

More recently, Lourenço et al. (2022) showed that intrarenal levels of ANG I were consistently elevated in cats with CKD, while ANG II levels were not. This could imply a differential regulation of RAAS components within the kidney compared to the systemic circulation.

For a comprehensive overview of the RAAS and its suppression in dogs and cats, please refer to the excellent review by Ames et al. (2018).

RAAS activation, vascular inflammation and remodeling: lessons from experimental models and human studies

Excessive activation of the RAAS plays a crucial role in vascular inflammation and remodeling. This is particularly relevant as the American Heart Association (AHA) has recently issued a comprehensive Presidential Advisory highlighting the intricate connections between cardiovascular diseases (CVD), CKD, type 2 diabetes (T2DM), and obesity (Ndumele et al., 2023). This integrated approach aims to redefine how these conditions are understood and managed, introducing the concept of *Cardiovascular-Kidney-Metabolic* (CKM) health which outlines the overlapping and mutually reinforcing risks of CVD, CKD, T2DM, and obesity. Among others, these diseases share common pathophysiological pathways, including inflammation, oxidative stress, and metabolic dysregulation, which exacerbate disease progression and complicate their therapeutic management overall.

Significant findings from the scientific literature on the role of RAAS activation in cardiovascular remodeling include, among others:

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- **Pro-inflammatory actions of angiotensin II**. ANG II regulates the expression of cytokines and chemokines in the kidneys, vessels, and heart, contributing to vascular inflammation and remodeling (Pacurari et al., 2014; Hahn et al., 1995; Tummala et al., 1999). Chronic infusion of ANG II is associated with increased BP, myocardial infiltration of inflammatory cells, and cardiac fibrosis (Qi et al., 2011).
- **Oxidative stress and end-organ damage**. ANG II-induced oxidative stress and mechanical injury from elevated BP result in end-organ damage, manifested by myocardial infarction, congestive heart failure (CHF), and CKD (Devonald & Karet, 2002; Chobanian et al., 2003).

This body of evidence explains why the classical pathway has historically been the primary focus of RAAS-mitigating drug therapy in both human and veterinary medicine. RAAS inhibitors, such as ACE inhibitors and angiotensin receptor blockers (ARBs) have been shown to significantly reduce levels of various pro-inflammatory markers, including C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α), and interleukins like IL-6 and IL-1β (Krysiak & Okopień 2011, 2012; Fernandez et al., 2008). These reductions in inflammatory markers contribute to the protective effects of RAAS inhibitors on cardiovascular and renal health. In particular, RAAS inhibitors reduce oxidative stress by decreasing the production of reactive oxygen species (ROS), thus preserving endothelial function and preventing vascular damage (Gainer et al., 1998). The anti-inflammatory effects of these inhibitors are mediated through the blockade of ANG II, which promotes inflammation and oxidative stress, and modulation of key signaling pathways such as NF-κB, which plays a critical role in the inflammatory response. Clinically, these effects translate to reduced cardiovascular morbidity and mortality, particularly benefiting patients with chronic inflammatory conditions such as heart failure, hypertension, and kidney disease. Additionally, the reduction in inflammatory markers associated with RAAS inhibitors improves outcomes in patients with metabolic syndrome and diabetes (Vaccari et al., 2008; Bähr et al., 2011).

A systematic review and meta-analysis of 32 randomized controlled trials consistently support these findings, underscoring the comprehensive benefits of RAAS inhibitors not only in blood pressure control but also in reducing systemic inflammation and protecting cardiovascular and renal function (Awad et al., 2022). This meta-analysis, which included 3,403 patients, found that ACE inhibitors significantly reduce inflammatory markers such as C-reactive protein (CRP), interleukin-6 (IL-6), and TNF-α. Specifically, ACE inhibitors such as perindopril and ramipril showed substantial reductions in IL-6, while enalapril reduced TNF-α and CRP. ARBs, however, showed a significant reduction in IL-6, but not consistently in other markers. These findings suggest that ACE inhibitors are more effective than ARBs in lowering systemic inflammation, highlighting their potential benefits in cardiovascular disease prevention and management.

A study published in the *European Heart Journal* (Cleland et al., 2021) recently reported the anti-inflammatory and anti-fibrotic effects of the MRA spironolactone in patients with heart failure. The study revealed that spironolactone significantly reduces pro-inflammatory cytokines such as TNF-α and IL-6, leading to decreased inflammation and fibrosis. This reduction also suppresses collagen type 1 synthesis, a key component of cardiac fibrosis. The study observed improved cardiac function, including enhanced left ventricular ejection fraction, and reduced ventricular remodeling. Clinically, spironolactone treatment was associated with fewer hospitalizations for heart failure exacerbations. These findings highlight the role of spironolactone in managing fluid retention, reducing inflammation, and limiting fibrosis, ultimately improving overall cardiac outcomes in heart failure patients.

Another mechanistic study published in *Clinical and Experimental Immunology* explored the anti-inflammatory and anti-fibrotic effects of spironolactone on *ex vivo*-activated human blood leukocytes through gene expression analysis and enzyme immunoassay (Bendtzen et al., 2003). This study focused on pro- and anti-inflammatory cytokines. Results showed a significant suppression of pro-inflammatory cytokine transcription and secretion, including TNF-α, lymphotoxin, IFN-γ, GM-CSF, and IL-6 (70–90% inhibition).

Finally, a study conducted by Kato et al. (2014) in RAW 264.7 macrophage-like cells and mouse peritoneal macrophages showed that spironolactone significantly inhibits the production of TNF-α

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and prostaglandin E2 induced by LPS. Overall, these results suggest that spironolactone would inhibit proinflammatory mediators induced by LPS through the inactivation of IκB kinase (IKK)/nuclear factor (NF)-κB.

Cardiovascular-kidney metabolic health beyond the RAAS: recent progress in the therapeutic management of cardiorenal diseases

Accumulating data from several large, placebo-controlled studies suggests that sodium-glucose cotransporter 2 (SGLT-2) inhibitors and glucagon-like peptide 1 (GLP-1) receptor agonists offer therapeutic benefits in the management of cardiovascular diseases, regardless of the patient's diabetic status (Zinman et al., 2015; Neal et al., 2017; Persson et al., 2018; Birkeland et al., 2019; McMurray et al., 2019; Inzucchi et al., 2020; Packer et al., 2020; Butler et al., 2021; Kosiborod et al., 2023).

In addition to their effects on glucose excretion, SGLT-2 inhibitors also have a positive impact on systemic metabolism by reducing systemic inflammation and oxidative stress, shifting metabolism towards ketone body production (Bertoccini and Baroni, 2021), promoting autophagy, and suppressing glycation end-product signaling. The SGLT-2 inhibitor dapagliflozin has been shown to have anti-inflammatory effects by inhibiting the overexpression of LPS-induced TLR-4 and activating NF-κB in human endothelial cells and differentiated macrophages (Abdollahi et al., 2022). In both human umbilical vein endothelial cells and macrophages, dapagliflozin significantly reduced the overexpression of TLR-4 under normal glucose and high glucose conditions. Furthermore, dapagliflozin shifted the balance from pro-inflammatory M1 macrophages to anti-inflammatory M2 macrophages (Abdollahi et al., 2022).

GLP-1 receptor (GLP1R) agonists are promising therapeutic agents due to their potent anti-inflammatory properties and diverse clinical implications. They regulate immune cell signaling, decrease activation of the NF-κB pathway, and lower levels of pro-inflammatory cytokines (Krasner et al., 2014; Wei et al., 2016). This modulatory effect leads to a decrease in systemic inflammation and an improvement in disease outcomes (Alharbi et al., 2024).

A study published by Cordeanu et al. (2023) in the *European Heart Journal* demonstrated a correlation between human cardiac GLP1R expression levels and low-grade inflammation and endothelial dysfunction. The study found that increased GLP1R expression leads to higher levels of oxidative stress, which can be reduced by GLP-1 receptor agonists through a canonical GLP1R AMPK-mediated pathway. Therefore, the cardiovascular benefits of GLP1RA may be attributed to their antioxidant and anti-inflammatory properties, particularly within the cardiac system.

A recent Phase IIIb clinical trial involving 3,533 patients with T2DM and CKD showed that patients who received the GLP1R agonist semaglutide were 24% less likely to experience "major kidney disease events", such as kidney failure or death due to kidney complications, compared to those who received a placebo (Perkovic et al., 2024; NCT03819153). Participants who received semaglutide were also 29% less likely to die from heart attacks and other major cardiovascular incidents than those who received a placebo, and 20% less likely to die from any cause during the trial period. The trial also showed that semaglutide may slow the deterioration of a person's kidneys. At 104 weeks, the urinary albumin-to-creatinine ratio was lowered by 12% in the placebo group, compared to 40% in the semaglutide group. Loss of kidney function, as indicated by the cystatin C-based eGFR, was lower by 3.39 mL per minute per 1.73 m^2 in the semaglutide group compared to placebo at that time. Based on experimental models and biomarker data, GLP1R agonists may have direct effects on the kidney, including reducing inflammation, oxidative stress, and fibrosis. The GLP1R is present in both the kidney (although moderately expressed) and immune cells. GLP1R agonists have been shown to decrease the expression of proinflammatory and profibrotic mediators in these cells (Alicic et al., 2021; Dalbøge et al., 2022; Tuttle et al., 2023; Alicic et al., 2023).

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Establishing relevant in vitro models to study anti-inflammatory effects of therapeutic drugs that modulate cardiovascular and kidney metabolic health

Conventional rodent models, such as mice, have been extensively used in studying human diseases due to their cost effectiveness, ethical considerations, and easy access to genetically engineered technology. However, despite the widespread use of murine models in biomedical research, the translational value of such studies remains limited because they often fail to accurately mimic human diseases (Jacob, 2016). A study by Seok et al. (2013), published in the *Proceedings of the National Academy of Sciences*, showed that mouse models poorly replicate inflammatory responses from different etiologies in humans. Among the genes that were significantly changed in humans, the murine orthologs had a very poor correlation (e.g., R^2 between 0.0 and 0.1) with their human counterparts.

Additionally, given the high failure rate of drugs during discovery and development, there is a critical need for more accurate animal models for preclinical studies (Waring et al., 2015). This need is highlighted by the recent passing of the FDA Modernization Act 2.0, which restricts the use of non-informative animal models, such as rodents, in testing experimental drugs before they can be evaluated in clinical trials (Zushin et al., 2023). This new act allows drug sponsors to use scientifically rigorous and proven non-model animal testing methods or replacement tests that are more suitable than animal testing.

As a result, there is now an incentive to use more relevant animal models, such as dogs, which are typically more representative for chronic disease modeling than rodents. This is because dogs have a relatively large body size, longer lifespan, physiological similarities to humans, and a tendency to develop spontaneous and clinical analogues of human diseases, such as cardiorenal and metabolic diseases, or other inflammatory and neurodegenerative diseases (Gordon et al., 2009; Gilmore & Greer, 2015; Jacob, 2016; Kaeberlein et al., 2016; Masters et al., 2018; Sebbag et al., 2018a,b, 2019, Sebbag & Mochel, 2020; Kopper et al., 2021).

Recent advances in biomedical research have led to the development of adult stem cells in three-dimensional culture systems, which enable *ex vivo* epithelial growth into organoids (Ootani et al., 2009, Sato et al., 2009). These stem cell-derived organoids offer several advantages over conventional two-dimensional epithelial systems, which utilize cancer-derived cell lines such as Caco-2, T84, and HT29 (Sun et al., 2004; Mochel et al., 2017), or spontaneously immortalized epithelial cells like rat intestinal epithelial cultures. These traditional methods can often fail to accurately reproduce the structure and function of the *in vivo* epithelium. In contrast, the 3D organoid culture method excels by better harnessing innate endogenous cellular programming within higher-order tissue organization (Foulke-Abel et al., 2014; Loewa et al., 2023).

Our laboratory has recently established an *in vitro* model using adult stem-cell derived organoids (Gabriel et al., 2022) stimulated by canine-specific TNF-α to study epithelial inflammation and drug response in dogs (Barko et al., 2024; Zdyrski et al., 2024). 3D organoids were dissociated and placed onto Transwell inserts to mimic cellular polarity. The 2D monolayers reached full coverage in about 8 days, with TEER values rising from around 19 $\Omega.cm^2$ on Day 3 to about 2,809 $\Omega.cm^2$ on Day 9. On the experiment day, 5 ng/mL of canine TNF-α was added to the basolateral chamber 4 hours after measuring TEER values and incubated for another 6 hours. This resulted in a significant decrease in TEER compared to pre-treatment levels. TNF-α also caused a significant increase in IL-8 secretion in both the apical and basolateral supernatants. RNA-sequencing showed that TNF-α treatment significantly upregulated various inflammatory cytokines and barrier function regulators (e.g., IL-7, IL-8, CXCL10, IL-17C, CCL20, IL-23A, ROCK1, ROCK2) in the canine colonoids.

Another recent study published by our laboratory (Sahoo et al., 2022) showed that LPS plays a significant role in promoting cancer progression by modulating gene expression and proliferation of intestinal epithelial cells in dogs with gastrointestinal diseases, such as inflammatory bowel disease (IBD) and intestinal mast cell tumors. The study concluded that LPS incubation leads to a pro-cancer gene expression profile and increased proliferation in IBD enteroids and colonoids. This study emphasized the significance of the crosstalk between the LPS/TLR-4 signaling pathway and various metabolic pathways, such as primary bile acid biosynthesis and secretion, the peroxisome, the renin-angiotensin system, glutathione metabolism, and arachidonic acid pathways. These interactions are critical in driving chronic inflammation and carcinogenesis.

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