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A Review of Relevant Therapeutic Targets for the Management of Congestive Heart Failure in Dogs: Present and Future Directions

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ABSTRACT

Congestive heart failure (CHF) is a major cause of morbidity and mortality with an increasing prevalence in human and canine populations. Similar to humans, overactivation of the renin-angiotensin aldosterone system is involved in the pathophysiology of CHF in dogs. Current therapeutic strategies for the management of canine CHF include the use of RAAS inhibitors, diuretics and inodilators. The present review summarizes data from our own research on the modulation of the renin-angiotensin cascade in dogs in dogs, together with new findings (including novel therapeutic targets) from the veterinary and the human literature.

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ABBREVIATIONS

ACE	Angiotensin-converting enzyme
AI	Angiotensin I
AII	Angiotensin II
ALD	Aldosterone
ARB	Angiotensin receptor blocker
ARNI	Angiotensin receptor neprilysin inhibitor
ASI	Aldosterone synthase inhibitor
AT1R	AII type 1 receptor
BP	Blood pressure
cGMP	Cyclic GMP
CHAT	Circadian hyper-amplitude-tension
CHF	Congestive heart failure
CKD	Chronic kidney disease
DCM	Dilated cardiomyopathy
EG	Empaglifozin
GR	Glucocorticoid receptor
HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
HT	Hypertension
MMVD	Myxomatous mitral valve disease
MRA	Mineralocorticoid receptor antagonist
NLME	Nonlinear mixed-effects

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NP	Natriuretic peptide
OM	Omecamtive mecarbil
PK	Pharmacokinetics
PD	Pharmacodynamics
PCP	Procollagen type I Carboxy-terminal Proteinase
RA	Renin activity
RAAS	Renin-angiotensin-aldosterone system
RI	Renin inhibitor
U2	Urocortin-2

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Pathophysiology of Congestive Heart Failure in Dogs

Congestive heart failure (CHF) is a major cause of morbidity and mortality with an increasing prevalence in human and canine populations (Guglielmini, 2003; George et al., 2014). It is widely estimated that 10% of dogs examined by veterinarians have some form of heart disease. The two most common acquired heart disorders in dogs are degenerative mitral valve disease (DMVD, also referred to as **MMVD**) and dilated cardiomyopathy (**DCM**). Within these diseases, it is estimated that approximately 30% of dogs with MMVD and 70-80% of dogs with DCM will eventually develop CHF (Borgarelli et al., 2008; Calvert et al., 1997), suggesting that up to 1 in 20 dogs may be affected by this clinical syndrome. Prognosis for CHF in dogs ranges from 6-14 months, depending on underlying disease and other patient and comorbid factors (O'Grady et al., 2008). MMVD is characterized by thickening and shortening of the atrioventricular valves, and affects about 75% of dogs over the age of 16 (Guglielmini, 2003). While MMVD has been recognized in dogs for over a century, histopathological and clinical studies have not been able to reveal its cause or why it occurs ten times more frequently in dogs than in humans (Borgarelli & Buchanan, 2012).

In humans, left ventricular ejection fraction (EF; derived as the ratio of the stroke volume and the end-diastolic volume) is used to define two types of patient populations with heart failure (HF): HF with *reduced* (< 40%) EF (**HFrEF**) vs. HF with *preserved* EF (**HFpEF**). This distinction is key as EF is an important prognostic factor in HF, and HFpEF patients (approximately 50% of HF cases worldwide) are known to respond differently to available therapies (Clevand and Clark, 2012).

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Essentially, HFpEF patients present with a degree with **diastolic dysfunction**, analogous to what is being described in dogs with **MMVD**. However, in humans, HFpEF is usually a primary diastolic dysfunction issue rather than a valvular disease causing volume overload, as seen in dogs with MMVD. At the other hand of the spectrum, HFrEF, also referred to as **systolic HF** is analogous to canine **DCM**, although DCM is primarily due to myocardial dysfunction rather than ischemic heart disease like in humans.

Noteworthily, HFpEF has been defined as a systemic syndrome, affecting multiple organ systems and rooted in immune dysregulation and systemic inflammation (Patel and Shah, 2019). Several comorbidities, including CBD, diabetes mellitus, obesity and other chronic inflammatory diseases have therefore been associated with HFpEF. This is important as the therapeutic management of HFpEF is geared towards integration of these various components. Importantly, there are currently **no approved drugs for the treatment of HFpEF**.

Similar to humans, the β-myosin heavy chain isoforms predominate in the dog myocardium (Hasenfuss, 1998), such that the excitation/contraction coupling in the myocardium of dogs appears to be similar to that in the human myocardium. More importantly, the pathophysiological cascade of renin activation, as observed in the course of CHF, is similar between dogs and humans, which motivated the choice of this animal species in the experimental work on the renin-angiotensin-aldosterone system (RAAS) and blood pressure (BP) pioneered by Guyton, Hall and co-workers (Cowley & Guyton, 1972; Guyton et al., 1972; McCaa et al., 1975; Young & Guyton, 1977; DeClue et al., 1978; Lohmeier et al., 1978; Hall et al., 1980, 1984; Wilczynski & Osmond, 1983). Renin release from the juxtaglomerular apparatus is a common compensatory mechanism to

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the reduced cardiac output observed in symptomatic stages of canine and human heart failure (Watkins et al., 1976; Hall, 1991). Recognition of the dysregulation of the RAAS in the pathophysiology of CHF has led to significant medical advances (McMurray et al., 2012). Reduction of angiotensin II (AII) and aldosterone (ALD) levels is paramount to prevent life-threatening complications associated with myocardial fibrosis and systemic hypertension.

An Overview of the Renin-Angiotensin Aldosterone System: Past and Present

A Complex and Highly-Regulated Machinery

Various authors have amply reviewed the role of the RAAS in the regulation of BP and volume homeostasis (Ferrario & Strawn, 2006; Moon, 2013; Sayer & Bhat, 2014). The expression of certain RAAS components even in simple organisms like crustaceans, insects and leeches underscores the importance of the renin cascade in the control of cell volume and water homeostasis throughout evolution (De Mello, 2014). The history of the RAAS and its discovery has recently been retraced with great accuracy in a review paper by Tsukamoto & Kitakaze (2013).

A common description of the functioning of the systemic RAAS cascade begins with the release of renin from granular cells of the juxtaglomerular apparatus, in response to changes in sodium chloride concentrations, decreased renal blood flow, and sympathetic stimulation. Many studies have established that renin secretion is inversely related to renal perfusion pressure (Hackenthal et al., 1990; Bock et al., 1992), while β-adrenergic activation has been shown to stimulate renin release in several species, including the dog (Lew & Summers, 1987). **Renin** catalyzes the conversion of the precursor angiotensinogen to **angiotensin I** (AI), which in turn is converted to the **octapeptide AII**

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by the angiotensin-converting enzyme (ACE) as it passes through the pulmonary capillaries. Enzymes other than ACE may contribute to the conversion of AI to AII. Chymase, cathepsin G, tonin and other proteases have been described as alternative pathways of AII production (Weber et al., 1995; Roig et al., 2000). AII is a potent vasoconstrictor with additional endocrine (e.g. ALD and arginine vasopressin secretion), neuronal (e.g. sympathetic noradrenaline release), and renal (e.g. glomerular filtration rate modulation) actions (Tsukamoto & Kitakaze, 2013). The majority of these effects are mediated through selective binding of AII to AT_1 receptors. In most cases AT_2 receptors binding elicits vasodilation, but cardiomyocyte hypertrophy and cell death have also been reported with stimulation of AT_2 receptors (Henrion et al., 2001). **Aldosterone** secretion from adrenocortical cells of the zona glomerulosa contributes to body fluid and acidobasic homeostasis via sodium, potassium and hydrogen ion exchanges in the distal renal tubules and collecting ducts of Bellini (Quinn & Williams, 1988). Note that the effect of ALD on the regulation of natriuresis and BP would be quantitatively less important than the action of AII on proximal tubular sodium reabsorption. This direct intrarenal effect of AII further results in reduced urinary flow in the tubular segments of the medulla, thereby increasing medullary osmolality and fluid reabsorption in the descending loop of Henle and the collecting ducts of Bellini (Hall, 1991).

Next to the systemic (circulatory) renin cascade, several RAAS components are also produced at the tissue level, in the heart, the vascular endothelium, or the kidneys (Danser, 1996; Danser et al., 1997). This ‘local RAAS’ functions as an autocrine or paracrine system and regulates tissue growth and repair processes. It is now recognized that the conventional renin/ACE/AII/AT_1 cascade is no longer the sole signaling pathway

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of the RAAS. At least 3 new axes have recently been identified in the kidneys and other tissues (Zhuo et al., 2013). These include: i) the **ACE2/ANG$_{(1-7)}$/Mas** receptor pathway, that may play an opposing role to the **renin/ACE/AII/AT$_1$** axis (Esteban et al., 2009), ii) the prorenin/PRR/MAP kinases ERK1/2 axis, which appears to be pivotal in the development of diabetic nephropathy in rodents (Ichihara et al., 2004, 2006), and iii) the ANGIV/AT$_4$/IRAP cascade, whose implication in the regulation of BP and renal modulation remains controversial. With the discovery of these additional pathways, the action of the RAAS has been extended beyond the regulation of BP, sodium and fluid homeostasis by the AT$_1$ receptor.

RAAS Activation in Vascular Inflammation, Remodeling and Congestive Heart Failure

Excessive activation of the RAAS plays an essential role in vascular inflammation and remodeling (Pacurari et al., 2014). Animal and human studies have shown that AII possesses pro-inflammatory actions by regulating the expression of cytokines and chemokines in the kidneys, vessels and the heart (Hahn et al., 1994; Tummala et al., 1999). Consequently, chronic infusion of AII has been associated with increased BP, myocardial infiltration of inflammatory cells, and cardiac fibrosis (Qi et al., 2011). Many of these pathophysiological changes can be attributed to mechanical injury from elevated BP and AII-induced oxidative stress (Weir, 2006), and will eventually result in end-organ damage manifested by myocardial infarction, CHF, and chronic kidney disease (CKD) (Chobanian et al., 2003). The pro-inflammatory and pro-fibrotic effects of the RAAS are also mediated by ALD, which further promotes insulin resistance and vascular remodeling (Martinez, 2010; Cascella et al., 2010).

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While the relation of systemic hypertension (HT) to the development of CKD has not been extensively documented in small animals, there is reasonable evidence to justify extrapolation of these considerations from human to dog patients (Lefebvre et al., 2007). In humans, the degree of activation of the renin-angiotensin aldosterone cascade is related to the severity of heart failure (Swedberg et al., 1990; MacFadyen et al., 1999). In this population of patients, AII concentrations vary from less than 10 pg/mL in mild cases of CHF, to 70 pg/mL in seriously affected individuals (Van de Wal et al., 2006). AII is viewed as a primary determinant of end-organ damage (Roig et al., 2000), while ALD is known to worsen AII tissue-damaging properties (Rocha et al., 1999). Thereof, elevated exposure to AII and ALD has been associated with a poor prognosis in multiple case studies (Roig et al., 2000; Latini et al., 2004). Swedberg et al. (1990) have found a positive correlation between mortality and levels of AII ($P < 0.05$) and ALD ($P < 0.003$) in a group of severe CHF patients. More recently, a 12 months follow-up study showed that AII was a significant predictor of death or new heart failure episodes in patients with left ventricular dysfunction (Roig et al., 2000). Likewise, high ALD concentrations were found to be a predictor of increased mortality risk that provides complementary prognostic value in a prospective cohort experiment of 294 patients with CHF of any cause and severity (Güder et al., 2007).

Compared with the depth of data from the human literature, only limited information on the relation of AII and ALD to a morbidity and mortality risk is presently available in dogs. Knowlen et al. (1983) have established a direct relationship between ALD and the clinical status of dogs suffering from heart failure. Results from Bernay et al. (2010) in a multicenter prospective trial indicate that ALD receptor antagonism decreases the risk of

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cardiac death, euthanasia, or severe worsening in dogs with moderate to severe MMVD. Ovaert et al. (2010) suggest that patients with elevated AII and ALD could benefit from additional therapy with AII receptor blockers (ARBs), or MRAs. However, ALD escape has also been reported during long-term use of ARBs and MRAs (Naruse et al., 2002; Rousseau et al., 2002). In a study by Naruse et al. (2002), ALD increased above pre-treatment levels after 8 weeks of ARB administration, causing end-organ damage and left ventricular hypertrophy in rodents. In addition, results from the RALES Neurohormonal sub-study (Rousseau et al., 2002) showed a significant increase in AII and ALD over time ($P = 0.003$ and $P = 0.001$, respectively) in spironolactone-treated CHF patients.

ACE Activity is not a Reflective Measure of RAAS Suppression

ACE inhibitors have constituted a breakthrough therapeutic option in the management of cardiovascular diseases in human and veterinary patients (Pfeffer et al., 1992; BENCH Study Group, 1999). Earlier investigations on the use of benazepril in dogs have established that benazeprilat produces a complete and long-lasting inhibition of ACE. In a study by King et al. (1995), oral administrations of benazepril (0.25 mg/kg q24 h) were responsible for more than 85% inhibition of ACE during 24 hours. In addition, Toutain and Lefebvre (2004) have shown that an oral daily dose of 0.125 mg/kg benazepril causes inhibition of the entire systemic ACE pool within 48 hours.

However, results from our group (Mochel et al., 2013a, Mochel et al., 2015) demonstrate that benazeprilat triggers a marked fall in AII and ALD, but for a much **shorter period of time**, which is consistent with earlier observations in human patients (Lijnen et al., 1982; Jorde et al., 2002). According to Van de Wal et al. (2006), 45% of severe CHF patients experience **elevated AII levels independent of serum ACE activity**. In individuals with

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high ACE activity, non-compliance should be considered along with inadequate dose selection as potential explanations. Yet, in patients with low measurable ACE activity, this could be related to the production of AII by up-regulation of ACE independent pathways (Fyhrquist and Saijonmaa, 2008), in response to renin activation and accumulation of AI during short and long-term use of ACE inhibitors (Geary et al., 1992). Enzymes other than ACE may contribute to the conversion of AI to AII. **Chymase**, cathepsin G, tonin and other proteases have been described as alternative pathways of AII production (Roig et al., 2000). Our findings on ALD are also in agreement with earlier reports from the veterinary (Knowlen et al., 1983; Koch et al., 1994) and human literature (Lijnen et al., 1982; Cleland et al., 1984). Because AII is a known driver of ALD biosynthesis (McCaa et al., 1980), the partial suppression of AII in ACE inhibitor-treated dogs may account for the insufficient suppression of systemic ALD levels. Moreover, the possibility of an enhanced sensitivity of the adrenal glands to AII during chronic ACE inhibitor usage cannot be discarded (Lijnen et al., 1982). Finally, like all ACE inhibitors, benazeprilat has the potential to induce natriuresis and potassium retention, which can further stimulate secretion of ALD from the adrenals.

Role of Cortisol in Disease Development

Cortisol is an endogenous glucocorticoid secreted in conditions of physiologic or pathologic stress or inflammation. Most of cortisol's physiologic actions are **genomic effects mediated by binding to intracellular glucocorticoid receptors** (**GRs**). Effects of GR stimulation on metabolic and immune pathways allow the body to withstand stress and inflammation. Specific functions of glucocorticoids include stimulation of gluconeogenesis, mobilization of protein and fat stores, stabilization of lysosomal

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membranes and capillary walls, and decreased migration or function of white blood cells and other immune system components.

Although aldosterone is typically considered the "target ligand" for MRs, **cortisol actually binds MRs with the same affinity as aldosterone**, and circulating concentrations of free cortisol are 100-200 times higher than aldosterone (Levine et al., 1982; Broqvist et al., 1989). In healthy patients, cortisol simply occupies the MR binding site without activating the receptor. In non-renal tissues, such as the heart and vasculature, this tonic inhibitory binding capability is conferred by the enzyme 11β-hydroxysteroid dehydrogenase type II (11βHSD2) (Aronson, 2003). However, in inflamed or hypoxic tissues, 11βHSD2 function is impaired by abnormal oxidation-reduction potential, and **cortisol is able to activate MRs and mimic the actions of aldosterone** (Ettinger et al., 1998; Dooley et al., 2012).

In humans with chronic CHF, both cortisol and aldosterone are independent and complementary predictors of increased mortality, with high levels of *both* hormones associated with the worst prognosis (Güder et al., 2015). Another study of humans with acute decompensated CHF demonstrated that the prognostic value of these biomarkers depended on whether patients were receiving MRBs (Tidholm et al., 2005). In patients not receiving MRBs, both aldosterone and cortisol were again independent and incremental predictors of outcome. However, in MRB-treated patients, only aldosterone remained a significant predictor of mortality; cortisol was no longer associated with outcome (Tidholm et al., 2005). These findings suggest that the **pharmacologic benefit of blocking MRs** may have more to do with **blocking cortisol** than with blocking aldosterone, and that measures of RAAS activation (such as aldosterone levels) alone

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may have limited value in determining whether a patient will benefit from MRBs. While previous studies have established the prognostic value of cortisol in human CHF, the effects of endogenous cortisol levels in canine CHF remain unknown.

Established Pharmacological Targets in the Treatment of Canine CHF

Inhibition of the RAAS, as part of a global therapeutic scheme to decrease AII and ALD exposure, and to lower BP for preventing, or delaying end-organ damage, has proved to be effective in human and canine CHF (Chobanian et al., 2003; Lefebvre et al., 2007). Among RAAS inhibitors, two classes of drug directly target AII through complementary modes of action: i) **ACE inhibitors** prevent the formation of AII and the degradation of bradykinin, which increases the stimulation of nitric oxide and has positive effects on endothelial function, while ii) **Angiotensin Receptor Blockers** (ARBs) selectively antagonize AII at AT_1 receptors. A theoretical advantage of ARBs lies in their ability to increase activation of the AT_2 receptor, and modulate the effects of AII breakdown products (Liu et al., 1997), while reducing the risk of ALD escape. In practice though, an escape phenomenon has also been reported during long-term use of ARBs. In a study by Naruse et al. (2002), ALD increased above pre-treatment levels after 8 weeks of ARB administration, causing end-organ damage and left ventricular hypertrophy in rodents. Although non-peptide ARBs have found extensive applications in the treatment of cardiovascular disorders in human medicine, their use in small animal patients has proven ineffective (Adams, 2009).

By decreasing systemic vascular resistance, ACE inhibitors are known to improve cardiac hemodynamics and exercise capacity in human and dog patients (Levine et al., 1984; Uretski et al., 1988; Lefebvre et al., 2007). Benazepril, enalapril, imidapril, and ramipril

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are currently approved for use in dogs with CHF. Of note, multiple studies have shown that imidapril was non-inferior to enalapril and benazepril (Amberger et al., 2004; Besche et al., 2007). Benazepril hydrochloride (Fortekor®; Novartis Animal Health, Basel, Switzerland), is a non-sulfhydryl prodrug which is converted *in vivo* by esterases into its active metabolite, benazeprilat, a highly potent and selective inhibitor of ACE (Webb et al., 1990) with well-documented effectiveness in symptomatic canine CHF (King et al., 1995; Lefebvre et al., 2007). In the BENCH Study (1999), the mean survival time of benazepril-treated dogs with mild to moderate CHF was improved by a factor of 2.7, as compared with the placebo group (428 vs. 158 days). A significant gain in exercise tolerance and clinical condition was also reported after 28 days of treatment. The favorable outcome of most CHF canine patients under ACE inhibition therapy, despite a potential incomplete reduction in AII and ALD, suggests that ACE inhibitors exert additional beneficial effects than AII suppression in the course of heart disease (The CONSENSUS Trial Study Group, 1987; Pfeffer et al., 1992). As pointed out by Brown and Vaughan (1998), inhibition of bradykinin degradation, which results in a subsequent gain in left ventricular relaxation and systolic dysfunction, may account for the clinical effectiveness of ACE inhibitors. Along with its effect on ACE inhibition and bradykinin degradation, the blood pressure-lowering action of benazepril could also drive part of the reported clinical efficacy. Cardiac remodeling is a known deleterious consequence of arterial hypertension (Azibani et al., 2012), and benazepril (2 mg/kg q24 h P.O, for 2 weeks) has been shown to reduce blood pressure significantly ($P < 0.05$) in a dog model of renal hypertension (Mishina and Watanabe, 2008).

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While the use of ACE inhibitors in symptomatic stages of CHF is well-accepted, data supporting their use in asymptomatic stages (ACVIM A and B) are more sparse. In a study by Kvart et al. (2002), long-term treatment with enalapril (0.25-0.5 mg q24h P.O) in 229 Cavalier King Charles with asymptomatic MMVD did not delay the onset of heart failure. Yet, another prospective study by Atkins et al. (2007) in 124 dogs (23 breeds) with MMVD (ACVIM Stage B2) did show a trend toward benefit in time to onset of CHF (primary endpoint, $P = 0.06$) and a significant improvement in all-cause mortality ($P < 0.02$) with enalapril treatment (0.45 mg/kg q24h P.O). Finally, the positive effect of ACE inhibition in preclinical MMVD (ACVIM Stage B1) was further supported by a retrospective study from Pouchelon et al. (2008). Likewise, benazepril was shown to significantly delay the time to onset of overt DCM in a retrospective analysis by O'Grady et al. (2009) including 91 Doberman Pinchers.

As opposed to ACE inhibitors, a great body of data has accumulated over the years to support the use of the inodilator **Pimobendan**, a selective inhibitor of phosphodiesterase 3, in preclinical stages of heart failure. The recently completed EPIC study enrolling 360 dogs with Stage B2 MMVD showed that chronic administration of pimobendan significantly delayed the preclinical period ($P = 0.0038$) as compared with placebo (1228 days vs. 766 days). Of note, the effect of pimobendan vs. enalapril in symptomatic stages of MMVD and DCM was compared in a pivotal double-blinded trial from the FDA (FDA, 2007). No apparent differences in the primary endpoint (treatment success) were reported between study groups and the estimated mortality (14% death) was identical between pimobendan and enalapril. Another study (QUEST) by Haggstrom et al. (2008) comparing pimobendan (0.4-0.6 mg/kg q24h P.O) and benazepril (0.25-1 mg/kg q24h P.O) in 226

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dogs with MMVD found a modest benefit in survival in dogs receiving the inodilator (hazard ratio = 0.688, $P < 0.01$).

More recently, **Mineralocorticoids Receptor Antagonists** (MRAs) have also been registered for use in canine patients suffering from CHF. Although Schuller et al. (2011) could not find any significant effect of low-dose spironolactone (0.5 mg/kg q24h P.O) on survival when used as adjunct treatment to conventional CHF therapy, a subsequent study by Bernay et al. (2010) did show a significant reduction in risk or cardiac morbidity and mortality with the use of higher spironolactone dosage (2 mg/kg q24 h, P.O). In this study, spironolactone reduced by a factor of ca. 2 the risk of cardiac-related death, euthanasia, or severe worsening when used in addition to conventional therapy (ACE inhibition, plus furosemide and digoxin if required) in dogs with MMVD. These results were however disputed by Kittleson & Bonagura (2010) on the grounds of possible methodological flaws such as bias in patient categorization. In humans, MRAs have been associated with a significant reduction in mortality in human CHF patients when combined with ACE inhibitors, whereas ARBs have not (Werner et al., 2010). These positive outcomes support the current recommendation of the use of MRAs in the treatment of human CHF with reduced ejection fraction (Butler et al., 2012).

In a study by Chen et al. (2016) in humans with diastolic heart failure (NYHA Grade 1 and 2), spironolactone (40 mg q24h P.O) significantly improved clinical symptoms when associated with low-dose furosemide (20 mg q24h P.O). **Furosemide** is a cornerstone in the treatment of heart failure in human and veterinary medicine, but its use is typically associated with a significant elevation of ALD levels (Mochel and Fink, 2012). The positive effect of combined furosemide/spironolactone could therefore be related to the direct

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receptor antagonism of ALD in the context of RAAS activation. **Torasemide** (also referred to as torsemide) is a recently developed loop diuretic with a more potent and long-lasting effect than furosemide (Uechi et al., 2003; Hori et al., 2007). In addition, results from the TORIC study in humans with CHF demonstrated the superiority of torasemide over other diuretics (including furosemide) on patient mortality (Cosin et al., 2002). In a short-term term clinical trial of 366 dogs with MMVD (TEST study), Chetboul et al. (2017) showed that torasemide (0.24 mg/kg q24h P.O) was associated with a 2-fold reduction in risk of reaching a composite cardiac endpoint (spontaneous cardiac death, euthanasia due to heart failure or CHF class worsening) as compared with furosemide ($P < 0.05$). Results from Lopez et al. (2004) suggest that torasemide, but not furosemide, significantly reduce myocardial fibrosis; a mechanism that they later attributed to a reduction of PCP (Procollagen type I Carboxy-terminal Proteinase) activation, an enzyme involved in Collagen type I formation (Lopez et al., 2007).

Future Directions

Chronopharmacotherapy: Making the Best Use of Available Drug Therapies

Deeper understanding of circadian rhythms can have a substantial impact on the therapeutic management of RAAS-related diseases by determining the time of drug administration that would optimize efficacy while minimizing the occurrence of adverse effects. This concept, referred to as chronotherapy, is currently being used for the treatment of human rheumatoid arthritis (Staessen et al., 1992), lung cancer (Mazzoccoli et al., 2012) and cardiovascular diseases (Nicholls et al., 1993). An increasing number of investigations on the use of ACE inhibitors in hypertension have shown a greater reduction of BP with bedtime administration as compared with morning dosing (Palatini

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et al., 1992; Hermida & Ayala, 2009). Sole and Martino (2009) have demonstrated that heart and vessels growth and remodeling were dynamic and occurred more actively during the period normally allocated to sleep. In mice, administrations of the ACE inhibitor captopril at sleeping hours significantly improved cardiovascular function and reduced adverse remodeling, while no effects were reported when the drug was given during active hours of the day (Martino et al., 2011). In a study by Nozawa et al. (2006), temocapril (another ACE inhibitor) prolonged the survival of spontaneously hypertensive rats, with a maximum effect after dosing during the resting period, and a minimum effect after dosing at the active period. The authors concluded that treatment with an ACE inhibitor at night may be a more effective dosing regimen in patients with hypertension.

Another therapeutic approach in the management of heart failure and hypertension is to continuously assess not only the medical response, but also the development of adverse effects. The optimal treatment time can vary considerably between patients, as shown by the work of Watanabe et al. (2006, 2013) in hypertensive patients under losartan/hydrochlorothiazide (L/H) (angiotensin II receptor blocker/thiazide diuretics) combination therapy. In their study, L/H taken few hours before bedtime in a 61-year-old man induced circadian hyper-amplitude-tension (CHAT), a condition associated with an increased cardiovascular disease risk. For yet another patient, CHAT was exacerbated when L/H was given during the day, but was alleviated when the same dose of treatment was taken in the evening. In all instances, optimization of therapy based on the most appropriate time of drug administration should be investigated on an individual basis.

Until recently, no detailed information on the systems dynamics of the renin cascade was available in dogs. Research performed within our group presents the first description of

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the chronobiology of the canine RAAS in relation to BP, renal sodium/potassium handling, and feeding schedules using a NLME modeling approach (Mochel et al., 2013a, 2014). This model-based approach provided new insights into the relation of dietary sodium to RAAS chronobiology, which would have been impossible using standard statistics. Specifically:

i) ***The amount of sodium intake*** was shown to influence the tonic (i.e. mesor) and the phasic (i.e. amplitude) secretion of renin; the greater the intake of sodium, the smaller the mesor and amplitude of RA;

ii) ***The time of food (i.e. sodium) intake*** appeared to exert a synchronizing effect on the acrophase of RA and BP oscillations, which consolidates preliminary findings from the literature (Itoh et al., 1996).

Based on our findings on the dynamics of the circulating RAAS under physiological (Mochel et al., 2013a, 2014a), and RAAS-activated conditions (Mochel et al., 2013b, 2014b), various strategies could therefore improve therapeutic management of cardiovascular diseases in dogs. Essentially, one could think of:

i) ***Adjusting the time of dosing***. In dogs, cardioactive medications are commonly given with morning food for the sake of convenience. However, results from our chronobiological investigations with morning feeding indicate that the peak RA and BP occurs in the evening and at night. Assuming that drug efficacy is maximum when the peak effect time is synchronized with the peak of the underlying biological rhythm, one would expect **optimized efficacy with bedtime dosing** and morning feeding (or vice versa);

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ii) ***Adjusting dietary sodium intake***. Since high dietary sodium is thought to play a role in the development of HT, cardiovascular and renal diseases in humans, a common practice in veterinary cardiology was to restrict sodium intake in the diet of CHF dogs. There is however no substantial evidence that elevated sodium intake increases the risk of HT in dogs (see results from Anderson et al., 1986 and Greco et al., 1994 showing that fluctuations in sodium intake has no apparent effect on BP and heart rate), and the current recommendation is to avoid highly elevated dietary salt intake, without making a specific effort to restrict it (Chandler, 2008). Furthermore, because the mesor and amplitude value of RA oscillations was found to be much greater in dogs fed a low-sodium regime (Mochel et al., 2014b) we could assume that CHF dogs would rather benefit from a normal, not a restricted-sodium diet.

Taken together, our results suggest that additional research on the chronobiology of the RAAS is required in small animal patients to further improve therapeutic management of CHF in dogs by selecting the appropriate time of treatment.

Learning from Human Pharmaceutical R&D

Old Targets, New Drugs

Although spironolactone is relatively inexpensive, its use has been associated with multiple side effects in humans, including gynecomastia in men (Mosenkis and Townsend, 2004). This is due to the ability of spironolactone to bind to other steroid hormone receptors. To minimize the likelihood of such effects, more selective MRAs have been developed, such as **eplerenone** (2nd MRA generation) and **finerenone** (3rd MRA generation). The next generation will provide even greater selectivity towards the MR,

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while targeting select tissues to further improve the benefit-risk ratio of MRAs (Ames et al., 2019).

First generation **Renin Inhibitors** (RIs), such as aliskiren have shown disappointing results for the treatment of cardiovascular (ASTRONAUT and ATMOSPHERE trials) and renal diseases in humans (Gheorghiade et al., 2013; McMurray et al., 2016). The next generation of RIs is currently under development. Finally, previously developed **Aldosterone Synthase Inhibitors** (ASIs) lacked selectivity and were discontinued (Calhoun et al., 2011).

New Therapeutic Targets

1. Recently Approved Therapeutics: Sacubitril/Valsartan

Sacubitril/valsartan (Entresto®) is a first-in-class angiotensin receptor neprilysin inhibitor (**ARNI**), which upon oral administration delivers systemic exposure to sacubitril (AHU377) and valsartan, a well-established ARB recommended by established guidelines for the treatment of HF (McMurray et al., 2012; Langenickel & Dole, 2012; Yancy et al., 2013). Sacubitril is an inactive prodrug that is rapidly hydrolyzed by carboxyl esterase 1 to sacubitrilat, a pharmacologically active NEP inhibitor [23]. Lately, results of the Phase III PARADIGM-HF clinical trial comparing Entresto® with enalapril in patients with reduced ejection fraction CHF were disclosed in the New England Journal of Medicine (McMurray et al., 2014). Entresto® was found to be superior by ca. 20% to enalapril in reducing the risks of death and of hospitalization for heart failure ($P < 0.001$). Entresto® has now been approved in many countries for the treatment of HFrEF and is recommended by European and American HF guidelines (Ponikowski et al., 2016; Yancy

et al., 2016) for the treatment of chronic symptomatic HFrEF (New York Heart Association Class II–IV).

A preliminary dog study examined the effects of sacubitril/valsartan (225 and 675mg/day) vs. placebo, sacubitril (360mg/day), valsartan (900mg/day), and benazepril (5mg/day) on the dynamics of the renin-angiotensin-aldosterone system (RAAS) and the natriuretic peptide (NP) system in dogs. Beagle dogs (N = 18) were fed a low-salt diet (0.05% Na) for 15 days to model RAAS activation observed in clinical heart failure. Drugs were administered once daily during the last 10 days, while the effects on the RAAS and NPs were assessed on Day 1, 5, and 10 (Mochel et al., 2014, 2018). Compared with placebo, sacubitril/valsartan (675mg) substantially increased cGMP circulating levels, while benazepril and valsartan showed no effect. Additionally, sacubitril/valsartan (675mg) and valsartan significantly increased plasma renin activity, angiotensin I and angiotensin II concentrations. Finally, sacubitril/valsartan (both doses), and valsartan significantly decreased plasma aldosterone vs. placebo. Systemic exposure to valsartan following sacubitril/valsartan 675mg administration was similar to that observed with valsartan 900mg administration alone.

These results were later confirmed in a small prospective, randomized clinical study of sacubitril/valsartan (20 mg/kg q12h P.O) in 13 dogs with MMVD showing a significant reduction in urinary aldosterone to creatinine ratio vs. placebo (P = 0.032) (Newhard et al., 2018). These positive findings in dogs suggest that sacubitril/valsartan is a promising pharmacological candidate for increased survival in canine cardiovascular diseases.

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2. Drugs Showing Encouraging Results in Human Clinical Trials

The vast majority of ongoing clinical trials in human patients with heart failure are being conducted in HFrEF. Therefore, this paragraph exclusively focuses on current advances in this patient population. A list of novel pharmacotherapeutic modalities investigated in pre-clinical and clinical HFrEF studies is provided in Selim et al. (2017).

Omecamtive Mecarbil. Omecamtive Mecarbil (OM) is different from other inotropes as its mode of action is independent of Ca^{2+} intracellular increase. As such, OM has been shown to improve myocardial systolic function without a concomitant increase in oxygen consumption (Selim et al., 2017). In the COSMIC-HF Phase II, placebo-controlled trial including 448 patients with HFrEF, OM showed a concentration-dependent improvement in myocardial function (Teerlink et al., 2016). Launching of the Phase III program was announced in the fall of 2016.

Empaglifozin. Empaglifozin (EG) is an anti-diabetic medication that selectively inhibits the sodium glucose cotransporter 2 (Heise et al., 2013), while acting as an osmotic diuretic to reduce systemic BP (Tikkanen et al., 2015). A post-hoc analysis of the EMPA-REG OUTCOME trial looking at a subgroup of 706 patients with HF at baseline showed a significantly lower rate of cardiovascular death and HF hospitalization in type 2 diabetes patients receiving EG vs. placebo (Fitchett et al., 2016). A clinical trial is currently underway to investigate the effect of EG in CHF patients with or without diabetes.

SERCA2 Activator. SERCA2 is a specialized Ca^{2+} pump that is responsible for calcium reuptake in the sarcoplasmic reticulum. The CUPID study was designed to evaluate the efficacy of gene transfer using adeno-associated virus (AAV1) for delivery

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of SERCA2 cDNA in patients with HF (Jessup et al., 2011). A follow-up trial (SERCA-LVAD) is currently underway.

CD-NP. CD-NP is a synthetic NP causing vasodilation with minimal effect on BP. In addition, CD-NP has demonstrated an inhibitory effect on myocardial fibrosis in end-stage HF patients (Ichiki et al., 2014), as well as ALD production in healthy subjects (Lee et al., 2009).

Urocortin-2. Urocortin-2 (U2) is a member of the CRF (Corticotropin-Releasing Factor) family with a high affinity to the CRF receptor. U2 was shown to improve myocardial function in animal models of HF. A preliminary clinical trial of 53 patients with acute HF showed promising results (Chan et al., 2013), however larger studies in patients with chronic HF are warranted to further evaluate the benefit of U2 in CHF.

Conclusions

In conclusion, modulation of the renin-angiotensin aldosterone cascade remains the treatment of choice for management of chronic heart failure in human and veterinary medicine. Administration of therapeutic drugs at a time where they are most likely to be effective and/or best tolerated using chronobiological approaches has the potential to significantly increase the efficiency of RAAS inhibitors at no extra-cost. As shown in other therapeutic classes (Fink et al., 2012; Pelligand et al., 2016; Riviere et al., 2016; Lin et al., 2016; Bon et al., 2018), pharmacokinetic-pharmacodynamic modeling is an attractive tool to integrate the large body of information on RAAS physiology, regulation and modulation for the selection of relevant therapeutic doses (Hallow et al., 2014; Martinez et al., 2018). Canines have long been used for the preclinical testing of human

cardioactive drugs and represent an attractive spontaneous disease model to study innovative therapeutic strategies. In return, information on new therapeutic targets for CHF from human clinical trials can guide the development of future therapeutic candidates in veterinary cardiology, under the so-called 'One Health' initiative (Schneider et al., 2018).

Sacubitril/valsartan has recently been given Class I recommendation, the strongest endorsement, in updated clinical practice guidelines simultaneously released by the American College of Cardiology, the American Heart Association and the Heart Failure Society of America in the US. Guidelines now establish sacubitril/valsartan as standard of care for HFrEF. Preliminary efficacy and safety findings in disease models of RAAS activation and clinical patients are encouraging in dogs but deserves further investigation in larger patient cohorts. Another promising combination is the association of loop diuretics with aldosterone receptor antagonists, such as spironolactone. Given the proven benefit of torasemide over furosemide and the improved selectivity of the most recent MRAs, the combination of eplerenone and torasemide could be evaluated in dogs with CHF. Finally, positive findings from the COSMIC-HF program on myocardial function in HFrEF also positions Omecamtive mecarbil as an attractive target for the treatment of canine CHF.

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