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Summary in English

Dynamics of the Renin-Angiotensin Aldosterone System in Dogs:
Circadian variations in physiological conditions and in relation to
Angiotensin-Converting Enzyme inhibition
Congestive heart failure (CHF) is a primary cause of morbidity and mortality with an increasing prevalence in human and canine populations (Guglielmini, 2003; George et al., 2014). In both species, recognition of the overactivation of the renin-angiotensin-aldosterone system (RAAS) in the pathophysiology of CHF has led to significant medical advances (Sayer et al., 2009; 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. By decreasing systemic vascular resistance and AII production, angiotensin-converting enzyme (ACE) inhibitors have been shown to improve cardiac hemodynamics and reduce mortality in human and dog CHF patients (Levine et al., 1984; Uretski et al., 1988; Lefebvre et al., 2007). Although several experiments have pointed out the administration time-dependent efficacy of ACE inhibitors (Tata et al., 2005; Hermida & Ayala, 2009), little attention is commonly paid to the optimum time of dosing of these medications. A thorough characterization of the chronobiology of the renin cascade has the potential to streamline the therapeutic management of RAAS-related diseases in dogs, and help determining the time of drug administration that would maximize efficacy, while minimizing the occurrence of adverse effects.

The ambitions of the present research were threefold: i) first, to build a mechanistic representation of the chronobiology of the renin cascade in relation to blood pressure (BP) and renal sodium/potassium (Na/K) handling in dogs, ii) then, to evaluate the effect of various feeding patterns (i.e. dietary sodium intake and feeding time) on the periodicity of these systems, and iii) finally, to quantify the perturbations in dynamics of the systemic RAAS following inhibition of the angiotensin-converting enzyme using a nonlinear mixed-effects (NLME) modeling approach. The value of modeling and simulation lies in its ability to integrate all available information for understanding the functioning of a biological system, and its response to drug exposure under (patho-) physiological conditions.

A prerequisite of this work consisted in developing dedicated analytical methods for measurement of systemic RAAS levels in dogs. Circulating angiotensin I (AI) and AII concentrations were analyzed with validated EIA assays using specific monoclonal antibodies. Plasma renin activity (RA) was estimated by measuring the rate of AI formation after incubation of endogenous renin and angiotensinogen in plasma (2 h, 37 °C, pH 7.2). Aldosterone levels were finally quantified using a liquid chromatography–tandem mass spectrometry method with an isotope dilution technique.

Our research presents the first description of the chronobiology of the RAAS in relation to BP, renal Na/K handling, and feeding schedules in dogs using NLME models. It shows that RA, urinary ALD, BP, and urinary Na/K oscillate with a circadian periodicity in healthy trained and relaxed dogs. A combination of cosine and surge functions was able to describe and predict the periodic variations of the experimental data with good accuracy,
as indicated by the quality of the goodness-of-fit diagnostics. The use of mixed-effects models allowed borrowing information from the densely sampled plasma variables to improve parameters estimation of the (more sparse) urinary endpoints. Further, our model-based approach provided new insights into the relation of dietary sodium to the chronobiology of the RAAS and BP in dogs, which would have been impossible using standard statistics. First, the amount of sodium intake was shown to influence the tonic (i.e. mesor) and the phasic (i.e. amplitude) secretion of renin (i.e. the greater the intake of sodium, the smaller the mesor and amplitude of RA) (Mochel et al., 2013a, 2014a). Second, the time of food (i.e. sodium) intake appeared to exert a synchronizing effect on the acrophase of RA, urinary Na/K and BP oscillations. Introducing a 6 h or a 12 h delay in the dogs’ feeding schedule caused a shift of similar magnitude in the rhythm of these biomarkers, as confirmed by the model-based estimates of the phase shift parameter (Mochel et al., 2014a). Our results suggest that food intake provides cues that are able to act as synchronizers of the renin cascade, renal Na/K handling, and BP in dogs. Postprandial changes in RA would be related to sodium and water-induced body fluid expansion, while variations in urinary Na are reflective of the ‘impulse-response pattern’ of sodium excretion, which is characterized by a peak natriuresis 4 to 8 hours after feeding (Boemke et al., 1995). The marked post-meal drop in systolic and diastolic BP would be the result of reduced RA combined with the secretion of vasodilatory gut peptides, such as neurotensin and insulin (Shibao et al., 2007). Finally; although BP does not drop at night in healthy dogs fed a normal-sodium regime at 07:00 h, it does so in dogs fed at 19:00 h, indicating that similar to humans, lower BP levels are to be expected at nights when dogs are given an evening meal.

In dog CHF patients, ACE inhibitors are most often given with morning food for the sake of convenience. However, based on our findings on the chronobiology of the circulating RAAS and its relation to food intake, the efficacy of ACE inhibitors is expected to be greater with bedtime compared with morning dosing (i.e. at a time where the peak drug effect is synchronized with the peak of the underlying biological rhythm). In addition, although it has widely been perceived that CHF management should include dietary sodium restriction, there is no substantial evidence that elevated sodium intake increases the risk of hypertension in dogs. On the contrary, because the mesor and amplitude value of RA oscillations was found to be much greater in dogs fed a low vs. a normal sodium regime (Mochel et al., 2014a) it seems reasonable to assume that dogs suffering from CHF would rather benefit from a normal than a restricted-sodium diet.

In a following step, we have developed an integrated PK/PD model that adequately captures the disposition kinetics of benazeprilat, as well as the time-varying changes of systemic renin-angiotensin aldosterone biomarkers without, and with ACE inhibition therapy. This mechanistic representation provides a quantitative framework for better understanding the effect of ACE inhibition on the RAAS. Our data show that benazepril
at its recommended dose in dogs (0.25-1.0 mg/kg q24 hours) influences the dynamics of the renin-angiotensin-aldosterone cascade, resulting in a profound but temporary decrease in AII and ALD, while increasing RA for about 24 hours (Mochel et al., 2013b, 2014b). The model predicted no time delay between the dynamics of benazeprilat, RA, AII and ALD, which is an indication of a rapid turnover of RAAS biomarkers in plasma. Simulations at steady state benazeprilat peak concentrations revealed a 2 to 3-fold change in circulating RAAS levels, and a more prolonged effect on RA (at least 16 hours) compared with AII and ALD (between 5 to 10 hours). The apparent shorter duration of effect of benazeprilat on AII and ALD could be related to the production of AII by up-regulated ACE-independent routes in response to renin and AI accumulation.

The model presented herein is not a completed piece of work but is, instead, a starting point for future data integration. First, the model can be refined as additional data from several increasing doses of benazepril become available. Second, the model should be extended by including additional PK/PD information from other RAAS inhibitors with distinct modes of action, to document how the system behaves when blocking upstream and downstream components of the cascade. Third, as the model builds on new preclinical data, it should also be refined by including RAAS measures from the target dog population (i.e. symptomatic cases of CHF). Ultimately, the final full model could be used i) as a screening platform for selection of drug candidates, ii) for optimizing the dosing regimen (i.e. dose, dosing interval, and dosing time) of existing or new RAS inhibitors, and iii) to predict efficacy of future cardiovascular leads.

Overall, this mechanistic-based approach is expected to streamline the development of cardiovascular drugs in dogs, as well as to provide a versatile tool for preclinical investigations of human pharmaceuticals.
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