Aldosterone receptor antagonists – how cardiovascular actions may explain their beneficial effects in heart failure

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Historically, aldosterone receptor antagonists (ARA) have been classified as ‘potassium sparing diuretics’. However, the positive effect of spironolactone, the most extensively studied ARA, on morbidity and mortality observed in humans suffering cardiac insufficiency could not be explained by the renal effect of the drug alone, and a pivotal clinical study has led to extensive research. Many experimental studies have demonstrated that ARA have previously unexpected beneficial effects on the cardiovascular system including reduction in remodelling of the vascular smooth muscle cells and myocytes and improvement of endothelial cell dysfunction in heart failure. These effects improve vascular compliance and slow down the progression of left ventricular dysfunction and end-organ damage. Furthermore, aldosterone receptor blockade also restores the baroreceptor reflex, improving heart rate variability in heart failure in humans. Some of these effects have been demonstrated in dog models of cardiac disease and so justified further investigation of the potential benefit of ARA in dogs with congestive heart failure (CHF). Positive effects of spironolactone on morbidity and mortality appear to have been seen in studies conducted in dogs suffering from naturally occurring CHF. In addition, eplerenone has been shown to have benefits in canine models of heart failure. The precise mechanisms by which ARA produce these beneficial effects in dogs remain to be determined but this group of drugs clearly provide therapeutic actions out-with their diuretic effects.

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INTRODUCTION

Aldosterone receptor antagonists (ARA: spironolactone and eplerenone) compete with aldosterone for binding to mineralocorticoid receptors (MR) thereby inhibiting the action of aldosterone. MR receptors are not only localized in the kidney but also in the heart and blood vessels.

Aldosterone receptor antagonists counteract the retention of sodium and water and consequently reduce aldosterone-induced potassium loss. Thus, ARA have been classified historically as so called potassium sparing diuretics. Spironolactone was the first ARA to be introduced into human medical use and has for a number of years been used empirically by veterinary cardiologists to reduce the potassium loss in combination with loop diuretics in dogs suffering from CHF. In healthy dogs, repeated oral dose of spironolactone at 1, 2, 4 or 8 mg/kg for 8 days, has no effect on water and sodium diuresis. Thus, it is thought that the diuretic action of ARA is likely to be dependent on elevated concentrations of aldosterone (Jeunesse et al., 2007).

The beneficial effects of spironolactone on morbidity and mortality observed in the clinical trials in humans (Pitt et al., 1999), and the apparent effects observed in clinical trials in dogs (EMEA – European Medicines Agency – Veterinary Medicines, 2007) cannot be explained only by the potassium sparing diuretic effects of this drug.

In recent years, it has become increasingly apparent that aldosterone plays a pivotal role in many pathogenic processes involved in progression of heart failure to its end stage. Aldosterone influences the progression of heart failure through a variety of mechanisms, most notably those that lead to cardiac and vascular remodelling and endothelial cell dysfunction. Aldosterone appears to mediate these pathological processes as part of the renin-angiotensin-aldosterone system (RAAS) and is capable of upregulating the formation and action of Angiotensin II (AngII) within these target tissues. Furthermore, it is clear that aldosterone secretion in heart failure is not only dependent on AngII. An understanding of the mechanisms of action of aldosterone and how this mediator contributes to progressive...
cardiac and vascular dysfunction in the heart failure patient provides a compelling and rational case for the use of ARA in the management of heart failure.

This paper presents an overview of the literature on the effects of aldosterone and ARA on the myocardium, vasculature and autonomic nervous system activity and highlights a small number of studies where data in dogs are available.

**ALDOSTERONE**

Aldosterone is a mineralocorticoid hormone. In mammals, aldosterone is the principal regulator of sodium and potassium homeostasis (Na+ and K+ balance) and hence is central to the control of extracellular fluid volume and blood pressure (Tan et al., 1999; Lombes et al., 2000).

Mainly synthesized in the zona glomerulosa of the adrenal glands, aldosterone is released into the circulation to be carried to target organs and exert its action, mainly through binding to MR. In addition to the classical adrenal biosynthetic pathway, extra adrenal sites of local aldosterone production have been identified in the heart, the vascular system and in other tissues (brain, kidney, lung and liver) (Dell’italia et al., 1999; Silvestre et al., 1999; Lombes et al., 2000).

Mineralocorticoid receptors are found in the kidney, in cardiac myocytes, endothelial and smooth muscle cells of the vasculature (large vessels), but also in the colon, salivary and sweat glands, and in neurons of the central nervous system (Lombes et al., 2000; Magni & Motta, 2003).

Activation of MR receptors in the kidney (distal tubules and cortical collecting ducts) by aldosterone turns on a cascade of events that lead to a rapid increase in sodium reabsorption, subsequent water reabsorption, and potassium excretion via several mechanisms (Rocha & Williams, 2002).

**Aldosterone in heart failure**

Heart failure is a progressive and irreversible syndrome. In the first stages of the disease, a decrease in cardiac output leads to activation of compensatory mechanisms, including the sympathetic nervous system, the RAAS and other vasoreactive substances. The activation of these mechanisms (leading to hyperaldosteronism and high circulating concentration of AngII) results in tachycardia, vasoconstriction and sodium and water retention, increasing circulating blood volume which in turn restores cardiac output and maintains cardiac filling pressures. These compensatory mechanisms are initially beneficial as they can improve cardiac output but do so at the expense of increased workload for the heart which has to function at increased filling pressures (increased preload) and overcome increased peripheral resistance (increased after load). Initially, these compensatory mechanisms are balanced by the activation of counter-regulatory mechanisms that prevent excessive sodium and water retention (e.g. natriuretic peptides) and vasoconstriction (e.g. upregulation of the endothelial nitric oxide synthase system).

Progression of the disease and chronic activation of the compensatory mechanisms give rise to deleterious effects, overcomes the counter-regulatory mechanisms and results in a worsening of cardiac function. This, in turn, stimulates the compensatory mechanisms further, resulting in a vicious cycle with the appearance of clinical signs. Heart failure does not develop when the heart is injured but when counter-regulatory hemodynamic and neurohormonal mechanisms are overwhelmed or exhausted (Sisson & Kittleson, 1999; de Morais, 2000).

In dogs suffering from CHF, a two- or three-fold increase of the mean plasma concentration of aldosterone has been observed (Häggström et al., 1996; Tidholm et al., 2001) giving rise to a chronic state of hyperaldosteronism secondary to cardiac dysfunction. However, the pulsatile nature of aldosterone release, breed and diet (i.e. sodium and potassium intake) are important factors to consider as they result in large intra-individual variations. Population studies of plasma aldosterone concentration may provide worthwhile results, while values for individuals may be less meaningful (Gardner et al., 2007).

Measurement of plasma concentrations of aldosterone, AngII or plasma renin activity found in mixed venous blood may not necessarily clearly reflect activity of the local systems that can produce these hormones (i.e. myocardial and vascular RAAS). Borgarelli et al. (2001) and Barlucchi et al. (2001) showed that there is a local RAAS in canine myocytes.

**ALDOSTERONE RECEPTOR ANTAGONISTS**

In human medicine, spironolactone, was registered in 1960 as a potassium-sparing diuretic. Since then, many studies have demonstrated that spironolactone not only produces a rapid increase in sodium excretion, subsequent water excretion, and potassium reabsorption in the kidney but also has beneficial effects on the cardiovascular system (see below). The latter might explain the reduction of morbidity and mortality observed in human patients suffering heart failure (Pitt et al., 1999).

In human patients, therapy with spironolactone has been associated with side effects (such as gynaecomastia, impotence, mastalgia and hirsutism) related to the anti-androgenic properties of the drug. For this reason, the selective ARA eplerenone, which has a much lower affinity for the androgen receptor, has been developed. In humans, in terms of mineralocorticoid effects, 25 mg spironolactone is approximately equivalent to 50 mg eplerenone (Kalidindi et al., 2007). Eplerenone (Inspra®; Pfizer, New York, NY, USA) has been registered for use in human medicine since 2004 and is indicated to improve survival of stable patients with left ventricular (LV) systolic dysfunction (ejection fraction ≤ 40%) and clinical evidence of CHF after acute myocardial infarction.

Torasemide (Torasemide Sandoz®; Novartis, Bale, Switzerland) is a loop diuretic (Hori et al., 2007), registered in human medicine, which also appears to have a potassium sparing diuretic action, presumably through the antagonism of aldosterone. In the dog, however, although the diuretic actions of torasemide have been demonstrated (Uechi et al., 2003), the
precise mode of its potassium sparing diuretic effect has not been clearly characterized in the published literature. This agent will not be considered further in the present review.

There are published data of the use of eplerenone in canine models of human heart disease and failure where beneficial effects are demonstrated (Suzuki et al., 2002). As yet no data are available in spontaneous canine CHF.

Spironolactone has been registered in Europe since 2007 (Prilactone; Ceva Santé Animale, Libourne, France) for use in combination with standard therapy (including diuretic support, where necessary) for the treatment of CHF caused by valvular regurgitation in dogs. The anti-androgenic properties of spironolactone appear not to create clinical problems in dogs with CHF. In target species tolerance studies with spironolactone, reversible prostatic atrophy was seen in entire male dogs at the recommended treatment dose.

In some countries, some angiotensin converting enzyme (ACE) inhibitor approved for veterinary use have a contra-indication for use with potassium-sparing diuretics or a possible interaction between both drugs is mentioned. In absence of data, this was added when ACE inhibitors were registered during the 1990s, as a precaution as both drugs spare potassium and hyperkalaemia was feared. As is the case in human medicine, the combination of spironolactone with angiotensin converting enzyme-inhibitors (ACEI) has been demonstrated to be well-tolerated in dogs with CHF due to degenerative mitral valve disease; hyperkalaemia was not observed in the conducted field trials (EMEA – European Medicines Agency – Veterinary Medicines, 2007). Similar findings were observed by Thomason et al. (2007) in dogs presenting mitral valve disease.

Beneficial effects of ARA on the cardiovascular system

In addition to its classic anti-natriuretic properties, which can lead to hypokalemia, aldosterone, as a component of the RAAS, has other adverse effects that can contribute to the pathophysiology of heart failure. These effects include cardio-vascular remodelling and fibrosis and endothelial cell and baroreceptor dysfunction (Weber, 2001; Kalidindi et al., 2007; Parthasarathy & MacDonald, 2007).

Effects on the heart

Myocardial fibrosis, heart failure and survival time

Studies conducted in laboratory animals, in dogs and in human patients document the existence of myocardial fibrosis in heart failure (see Table 1). LV remodelling is complex, dynamic and time dependent, and varies with the aetiology of the initial myocardial injury (Jugdutt, 2003). This has been most frequently studied in the context of human myocardial infarction and the LV remodelling that occurs thereafter. Remodelling of the extracellular collagen matrix (ECCM) plays a major role in LV remodelling and an appropriate balance between formation and degradation of ECCM is required for this process to be effective. An imbalance in ECCM turnover can ultimately result in myocardial fibrosis. For instance, chronic LV volume overload, leading to eccentric hypertrophy, is finally associated with increased ECCM, collagen cross-linking, and fibronectin deposition. The development of myocardial fibrosis in human patients causes pathologic hypertrophy with abnormal myocardial stiffness, which leads to ventricular diastolic and systolic dysfunction and ultimately to progression of symptomatic heart failure (Silvestre et al., 1999; Struthers, 1999; Tan et al., 2004). Therefore, antifibrotic drugs that target excess ECCM might be a logical therapeutic approach.

In dogs, experimental models of mitral valve regurgitation (see Table 1; Stewart et al., 2003) cause acute changes in LV volume leading to rapid overload of the left ventricle. It is clear that for the left ventricle to dilate rapidly, collagen degradation needs to exceed its synthesis so that LV wall compliance reduces and

Table 1: Literature on the existence of myocardial fibrosis in heart failure (naturally occurring and experimental models)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Model system used</th>
<th>Observations</th>
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<tbody>
<tr>
<td>Brilla et al. (1995) &amp; Lijnen et al. (2003)</td>
<td>Many studies reviewed, mostly conducted on laboratory species</td>
<td>In the pressure or volume overloaded heart: hypertrophic remodelling of the myocardium, with growth of cardiomyocytes and cardiac fibroblasts. Myocyte growth: primarily dependent on ventricular loading. Effector hormones of the RAAS: primarily involved in promoting the structural remodelling of the myocardial collagen matrix. Reparative fibrosis in response to cardiac myocyte necrosis, also occurs. Consequence: increased myocardial collagen matrix reducing myocardial compliance and hindering its contraction.</td>
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<tr>
<td>Falk et al. (2006)</td>
<td>Clinical findings in 21 dogs with CHF and valvular disease in comparison with 22 dogs without CHF.</td>
<td>Significantly more arterial narrowing and fibrosis in the left ventricle of dogs with naturally occurring CHF and myxomatous mitral valve disease than in control dogs.</td>
</tr>
<tr>
<td>Falk et al. (2007)</td>
<td>Clinical findings in 58 dogs with naturally occurring CHF</td>
<td>Correlation between echocardiographic abnormalities and myocardial fibrosis. Degree of overall fibrosis in the myocardium associated with shorter survival time. LV remodelling, in volume overload secondary to mitral regurgitation. Reduced interstitial myocardial collagen content associated with an increase in LV end-diastolic diameter.</td>
</tr>
<tr>
<td>Stewart et al. (2003)</td>
<td>Experimental dog model (chordal rupture) of mitral valve regurgitation</td>
<td>High concentrations of serum markers of cardiac fibrosis (collagen serum markers) significantly correlated with a poor outcome.</td>
</tr>
</tbody>
</table>

CHF, congestive heart failure; RALES, randomized aldactone evaluation study; LV, left ventricular; RAAS, renin-angiotensin-aldosterone system.
ventricular sphericalization and wall thinning can occur (Janicki et al., 2006). However, in naturally occurring mitral valve disease, ventricular remodelling results in dilatation of the left ventricle but this is accompanied by an increase in ventricular muscle mass (eccentric hypertrophy). Recent publications do support the occurrence of myocardial fibrosis in CHF secondary to naturally occurring mitral valve disease (see Table 1; Falk et al., 2006, 2007).

Although there is to date limited available evidence, this appears to support the occurrence of myocardial fibrosis in dogs with mitral regurgitation due to naturally occurring mitral valve disease.

Role of aldosterone in the development of myocardial fibrosis and prevention by aldosterone-antagonists

The influence of aldosterone, as a component of the RAAS system, in the development of myocardial fibrosis has become clear over the last 10 years. However, the exact molecular mechanisms leading to aldosterone-induced fibrogenesis remain to be elucidated. The evidence that aldosterone plays an important role in myocardial fibrosis is demonstrated by experimental model studies conducted in rats and dogs and in human clinical patients (see Table 2). These studies have demonstrated that ARA (spironolactone or eplerenone) administration prevents fibrosis in animals or human patients where ACEI inhibitors or AngII receptor antagonists (ARB) have been given, a previously unrecognized pharmacological effect.

The combined use of ARA and ARB to provide the most effective means of counteracting the cardiac remodelling that occurs in human heart failure (and hypertension) is justified by the fact that both aldosterone and AngII can stimulate cardiac fibrosis by distinct pathways. In the RALES (randomized aldactone evaluation study) in humans, it was concluded that blockade of aldosterone receptors by spironolactone, in addition to standard therapy of a loop diuretic, an ACE inhibitor and usually digoxin, substantially reduced the risk of both morbidity and death among patients with severe heart failure (Pitt et al., 1999). The patients that were found to benefit most from spironolactone treatment were those with high circulating concentrations of procollagen markers at entry to the study, suggesting a cause and effect relationship between the decrease in marker concentration and the beneficial effect of spironolactone. Patients with low concentrations of the markers of ECM turnover not only showed no decrease in these markers but also benefited little from spironolactone treatment. Indeed, heart failure progressed slowly in these patients. Thus, limitation of the excessive ECM turnover may be one of the various extrarenal mechanisms contributing to the beneficial effects of spironolactone on morbidity and mortality in patients with CHF (Zannad et al., 2000).

Similar effects of spironolactone (15 mg/kg/day) on reduction of fibrosis have been demonstrated in a canine model of human heart failure, induced by chronic rapid ventricular pacing (Yang et al., 2008 – see Table 2). In that study, spironolactone decreased the inducibility and duration of AF (atrium fibrillation) (P < 0.05), as well as atrial fibrosis (P < 0.01). The authors concluded that spironolactone contributed to the prevention of AF in dogs, and that this action was related to reduction of atrial fibrosis and was thus independent of hemodynamics.

In conclusion, as cardiac muscle fibrosis is a chronic consequence of the heart failure syndrome (whatever the cause), one of the beneficial effects of spironolactone may be through the inhibition of the remodelling-fibrotic pathway within the myocardium.

Effects on the vessels

Aldosterone promotes structural and functional alterations (vascular remodelling and endothelial dysfunction respectively) in the vasculature, which lead to a loss of compliance of the arteries and increased peripheral resistance (vasoconstriction). The binding of aldosterone to MR, induces oxidative stress and subsequent inflammation, fibrosis and atherosclerosis. Aldosterone also upregulates various subunits of the reduced form of nicotinamide dinucleotide phosphate (NADPH) oxidase and induces the generation of reactive oxygen species (ROS), end-organ damage and occurrence of fibrosis. In vitro and in vivo studies showed that these deleterious effects of aldosterone on the vasculature could be attenuated by ARA (see Table 3). However, as in the myocardium, the interplay between aldosterone and AngII is complex and to reverse the reduced vascular compliance, endothelial cell dysfunction and oxidative stress induced by the neurohormonal changes associated with the heart failure syndrome may require concomitant inhibition of both pathways.

In human cardiac patients, improvement of endothelial dysfunction, reduction of oxidative stress and slowing down of vascular remodelling, leads to a decrease of peripheral resistance and an improvement of vascular compliance, both of which contribute to an improvement in organ blood flow and a reduction in the clinical consequences of heart failure.

Vascular remodelling

Studies conducted in rats demonstrate that aldosterone may mediate and potentiate, through MR activation, the hypertrophic effects of AngII, on endothelial and vascular smooth muscle cells. The increased media/lumen ratio and decreased vascular collagen density is partially reduced by spironolactone (see Table 3).

In human medicine, vascular pathology is an extremely important area of research because of the common and often devastating effects of coronary heart disease. Atheroma development commences with intimal thickening progressing to fatty streaks and ultimately atherosclerotic plaques. Although the dog is a good experimental model for cardiovascular research in general, it is not a useful species in which to study vascular pathology as differing pathways of lipid metabolism make it resistant to atheroma development. Consequently, vascular pathology has been poorly studied in the dog and almost completely ignored in dogs with naturally occurring cardiac disease. Nevertheless, in dogs with naturally occurring CHF, narrowing of intramural coronary arteries in the left ventricle...
Table 2. Evidence for the role of aldosterone in the development of myocardial fibrosis and its prevention by aldosterone-antagonists

<table>
<thead>
<tr>
<th>Reference</th>
<th>Animal model of human heart failure or human patient study</th>
<th>Observations</th>
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</table>
| Brilla et al. (1990) &     | Rat in vivo study using three models of hypertension:     | Compared with control cells, collagen synthesis increased significantly after incubation with AngII or aldosterone.  
| Brilla et al. (1995)       | Renovascular hypertension                               | AngII- or aldosterone-stimulated fibrogenesis is abolished by AT1 (angiotensin 1) or ARA respectively. 
|                           | Infra renal aorta banding                               | Remodelling not seen in either ventricle with infra renal aorta banding (where Ang II and aldosterone are normal) despite comparable systemic hypertension and left ventricular hypertrophy. 
|                           | Chronic aldosterone infusion                            | Conclusion: both effector hormones of RAAS (AngII and aldosterone) lead to collagen accumulation in the heart, each mediator stimulating fibrosis through a distinct pathway. |
|                           | Many studies reviewed, mostly conducted on laboratory   |                                                                                                                                                                                                              |
|                           | species                                                 |                                                                                                                                                                                                              |
| Lijnen et al. (2003)       | Many studies reviewed, mostly conducted on laboratory   | Accumulation of collagen in the heart during chronic activation of the renin-angiotensin-aldosterone  
|                           | species                                                 | Spironolactone used at 20 mg/kg/day  
| Mill et al. (2003)         | Rat in vivo study (myocardial infarction by coronary artery ligation) | AT1 receptor (activated by AngII) mainly mediates type 1 collagen deposition  
|                           |                                                        | Aldosterone receptor mainly mediates type III collagen deposition  
|                           |                                                        | Spironolactone (20 mg/kg/day for 1 month) significantly reduced the collagen content in LV muscle in MI group by mechanisms independent of loading conditions of the heart chambers.  
|                           |                                                        | Aldosterone potentiates the effects of AngII, in particular the stimulation of collagen synthesis (collagen type I and III)  
| Robert et al. (1999)       | Rat in vivo study: Aldo infusion and increased salt intake | Spironolactone (15 mg/kg/day; 1 week before pacing and throughout pacing period) decreases atrial fibrosis (quantified by Masson trichrome staining; \( P < 0.01 \)). |
| Yang et al. (2008)         | Dog model of heart failure                              | Spironolactone contributes to atrial fibrillation prevention in dogs with CHF  
|                           | (pacing – 220 b.p.m. for 6 weeks)                      | Control dogs: LV (left ventricular) end-diastolic and end-systolic volume increased significantly and EF (ejection fraction) decreased significantly  
|                           |                                                        | Eplerenone-treated (10 mg/kg twice daily, orally for 3 months) dogs: LV end-diastolic, end-systolic volume and EF remained unchanged.  
| Suzuki et al. (2002)       | In vivo dog model of heart failure (intracoronary microembolization to reduce LV ejection fraction to 30-40%) | Spironolactone decreases the circulating concentrations of BNP (brain natriuretic peptide) and NT-proANP (N-terminal portion of atrial natriuretic peptide), which are activated in heart failure.  
|                           |                                                        | Spironolactone treatment led to a reduction in extracellular matrix turnover (suggesting reduced fibrosis), as indicated by decreased serum procollagen concentrations. 
| Pitt et al. (1999),        | Human patients study – heart failure patients treated   | Patients with higher than the median plasma concentration at entry to the study showed a significant decrease in serum procollagen concentrations after 6 months of treatment whereas equivalent patients receiving placebo showed no change in these markers over the same time period.  
| Rousseau et al. (2002),    | with standard therapy – RALES (randomized aldactone evaluation study) | Conclusion: spironolactone leading to a 31% reduction in risk of death. Result was attributed to a lower risk of both death from progressive heart failure and sudden death from cardiac causes. Patients on spironolactone had a significant improvement in the clinical signs of heart failure. The clinical beneficial effects of spironolactone were associated with reductions in plasma markers of fibrosis  
| Zannad et al. (2000),      |                                                        |  
| RALES Investigators (1996) |                                                        |  
| Satoh et al., 2002         | Human patients study – endomyocardial tissue from chronic HF patients and control patients | CYP11B2 (aldosterone synthase) expression and the collagen volume fraction increased in myocardial tissue from HF patients  
|                           |                                                        | Expression highest in patients with lowest ejection fractions  
|                           |                                                        | Expression lower in the subset of HF patients treated with Spironolactone and an ACE inhibitor than in not treated patients  

CHF, congestive heart failure; LV, left ventricular; ARA, aldosterone receptor antagonists; RAAS, renin-angiotensin-aldosterone system; AngII, angiotensin II.
Table 3. Vascular remodelling, endothelial dysfunction and oxidative stress

<table>
<thead>
<tr>
<th>Reference</th>
<th>Model system used</th>
<th>Observations</th>
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<tr>
<td>Hatakeyama et al. (1994) &amp;</td>
<td>Cultured smooth muscle cells and endothelial cells from pulmonary artery</td>
<td>Aldosterone may mediate and potentiate, through MR activation, the</td>
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<td>hypertrophic effects of AngII on endothelial cells and vascular smooth muscle</td>
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<td>Xiao et al. (2000)</td>
<td>Rat aortic smooth muscle cells grown in culture</td>
<td>This model led to decreased carotid artery distensibility and increased</td>
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<td>vascular collagen density. Spironolactone (10 mg/kg/day for 30 days) reduced</td>
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<td>the stiffness of carotid wall material independently of blood pressure and</td>
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<td>wall stress</td>
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<td>Nehme et al. (2005)</td>
<td>In vivo, rat model of ischemic heart failure</td>
<td>Aldosterone is a critical mediator of L-NAME/AngII-induced vascular damage</td>
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<td>(fibrinoid necrosis) through mechanisms apparently independent of its effects</td>
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<td>on blood pressure</td>
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<td>Rocha et al. (2000)</td>
<td>Rat in vivo study; L-NAME-AngII-NaCl model of hypertension</td>
<td>Heart failure has been linked to an induction of oxidative stress</td>
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<td>Chronic aldosterone/salt treatment activates NADPH oxidase with 3-nitrotyrosine</td>
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<td>activation and NF-kb activation expressed by endothelial cells and inflammatory</td>
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<td>cells, leading to a proinflammatory/fibrogenic phenotype involving vascular</td>
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<td>and nonvascular sites of injury. Spironolactone (200 mg/kg p.o. daily)</td>
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<td>attenuates these responses</td>
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<td>Sun et al. (2002)</td>
<td>Uninephrectomized rats receiving Aldo/salt treatment</td>
<td>Aldosterone participates in the development of structural (remodelling) and</td>
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<td>functional (endothelial dysfunction) vascular alterations induced by AngII</td>
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<td>Spironolactone (25 mg/kg/day) partially reduces the increased media/lumen</td>
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<td>ratio and the impaired response to acetylcholine of resistance arteries in</td>
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<td>AngII-infused rats</td>
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<td>Aortic NADPH oxidase activity is increased by AngII and aldosterone and reduced</td>
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<td>by spironolactone</td>
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<td>Virdis et al. (2002)</td>
<td>Ex vivo rat study. Rats treated chronically with infusion of AngII or Aldo for 14</td>
<td>Aldosterone participates in the development of structural (remodelling) and</td>
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<td>days isolated and endothelium dependent responses studied</td>
<td>functional (endothelial dysfunction) vascular alterations induced by AngII</td>
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<td>AngII-infused rats</td>
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<tr>
<td>Falk et al. (2006)</td>
<td>In vivo, dogs with naturally occurring CHF</td>
<td>Dogs with naturally occurring myxomatous mitral valve disease have significantly</td>
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<td>more arterial narrowing in the myocardium, lung and kidney than control dogs</td>
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<tr>
<td>Pedersen et al. (2003)</td>
<td>In vivo, dogs (Cavalier King Charles Spaniels with mitral regurgitation)</td>
<td>Reduction in circulation concentrations of nitrate/nitrite at sub-clinical stages</td>
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<td>of naturally occurring heart disease, suggesting endothelial dysfunction develops</td>
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<td>early in the course of developing MR in dogs</td>
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<tr>
<td>Farquharson and Struthers</td>
<td>In vivo, human patients with heart failure – sub-study RALES</td>
<td>Aldosterone reduces NO bioactivity and correlates inversely with arterial</td>
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<td>(2000)</td>
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<td>Spironolactone (50 mg/day one month) improves endothelial dysfunction:</td>
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<td>improves bioactivity of NO and acetylcholine-mediated endothelium-dependant</td>
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<td>vasodilatation. It also attenuates AngII but not AngII-mediated vasoconstriction</td>
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CHF, congestive heart failure; RALES, randomized aldactone evaluation study; LV, left ventricular; NADPH, nicotinamide dinucleotide phosphate; AngII, angiotensin II.

and intima-medial thickening in the pulmonary artery (but not in the aorta) have been observed postmortem (Falk et al., 2006) suggesting that significant vascular remodelling may occur in clinical cases of canine cardiac disease. However, the role of aldosterone in vascular remodelling in dogs has not been studied so far.

Endothelial dysfunction and oxidative stress

Endothelial dysfunction is usually assessed as impaired acetylcholine-induced, endothelium-dependent relaxation. This has been demonstrated both in numerous experimental models and in human patients with heart failure (see Table 3). The alterations in endothelial function are associated with, and may be secondary to, oxidative stress with increased production of ROS via NADH (reduced form of nicotinamide dinucleotide)/NADPH oxidase activation (Virdis et al., 2002; Chai & Danser, 2006). Free radical production and the NADH oxidase pathway are up-regulated by both AngII and aldosterone. Aldosterone has also been shown to reduce nitric oxide (NO) bioactivity, which seems logical since oxygen free radicals normally serve to inactivate NO (Farquharson & Struthers, 2000; Struthers, 2004).

Nitric oxide, a vasodilator which inhibits platelet aggregation, is considered cardioprotective under various circumstances. Nitric oxide may also diminish mitochondrial respiration and cardiac oxygen metabolism with a resultant decrease in heart work and consequently, myocardial oxygen demand. Aldosterone-mediated abrogation of nitric oxide formation or activity would impair not only vascular but also myocardial function (Stier et al., 2002).

Methods of measuring endothelial cell function in canine patients have not been validated. Nevertheless, a reduction in circulating concentrations of nitrate/nitrite has been reported in dogs in the sub-clinical stages of naturally occurring heart disease (Pedersen et al., 2003). The assumption was that circulating nitrate/nitrite is reflective of endothelial cell production although the mechanism of this proposed endothelial cell dysfunction has not been investigated. However, data from other

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species suggest that the RAAS and in particular aldosterone might play a role.

Several authors (see Table 3) have demonstrated that endothelial cell dysfunction could be attenuated by using ARA in human patients with heart failure. Markedly improved endothelial function was shown in human patients with advanced heart failure, who were already on ACE inhibitor therapy, after 1 month with spironolactone treatment. There is also evidence that the selective aldosterone blocker eplerenone reduces the generation of oxygen-free radicals (Struthers, 2004).

Aldosterone-mediated effects leading to endothelial dysfunction may, in part, account for its vascular pro-fibrotic actions. Endothelial dysfunction could lead to microthrombi, tissue microinfarction and injury, with repair through fibrosis. Whether aldosterone produces fibrosis directly or whether it acts via a vasculopathy-induced injury in tissues is an intriguing and yet unanswered question, but some experimental studies suggest the latter (Rocha et al., 2000).

Thus, in humans the pathological effects of aldosterone could occur initially at the level of small coronary arteries and are manifested as vasculcytotic lesions associated with fibrinoid necrosis of the media and expansion of the perivascular space. It has been suggested that the cardiac fibrosis induced by aldosterone and other components of the RAAS is a secondary event in response to vascular cytokine activation, inflammatory damage, and consequent ischaemic and necrotic changes. Further investigation is necessary to better understand the role of aldosterone in the inflammatory damage induced in the heart.

**Effects on sympathetic and parasympathetic activity**

Several experiments have demonstrated the deleterious effect of aldosterone on parasympathetic activity and its inhibition by ARA. In human subjects, aldosterone halved the bradycardic response to an equivalent pressor stimulus, an effect which was independent of changes observed in sympathetic activity (Yee & Struthers, 1998). Restoration of the baroreceptor reflex by spironolactone could thus decrease the risk of ventricular arrhythmia in humans by restoring normal heart rate variability.

Wang et al. (1992) and Wang (1994) showed that in dogs with experimental heart failure aldosterone not only directly reduces baroreceptor discharge from the carotid sinus, restoring normal baroreceptor function, but also reduces the heart rate response to changes in blood pressure. These effects were observed with both acute and chronic perfusion of aldosterone (50–500 pg/mL) into the carotid sinus.

In a study of human patients with CHF, spironolactone (50–100 mg/day) reduced heart rate and improved heart rate variability. The observation that spironolactone improved heart rate variability in CHF patients is strong indirect evidence for aldosterone having parasympatholytic effects. Any aldosterone-induced reduction in parasympathetic activity could contribute to cardiac death (MacFadyen et al., 1997). This parasympatholytic effect of aldosterone is particularly poignant since the parasympathetic nervous system is thought to oppose the arrhythmogenic effects of the sympathetic nervous system.

Neurohormonal dysfunction, as measured by heart rate variability, is a strong independent predictor of mortality in chronic heart failure in humans (Davies et al., 2005). In dogs suffering from idiopathic dilated cardiomyopathy, heart rate variability using the Vasovagal Tonus Index has been shown to be a useful prognostic tool (Pereira et al., 2008). The role of aldosterone in influencing heart rate variability and the significance of the latter in canine patients has not been investigated in dogs with naturally occurring CHF.

Finally, aldosterone potentiates the effects of catecholamines. In studies in rats, it was shown that aldosterone blocks the noradrenaline uptake in heart in vivo (Barr et al., 1995). The authors also showed, using Iodine 123-labelled meta-iodobenzylguanidine scanning, that spironolactone increased myocardial noradrenaline uptake in human patients with CHF. It is tempting to suggest that because catecholamines are arrhythmogenic, their potentiation by aldosterone may increase arrhythmia and hasten death in humans with heart failure (Struthers, 1999; Davies et al., 2005).

**CONCLUSION**

Aldosterone receptor antagonists have been thought of as potassium-sparing diuretics for many years. Recent major advances in the understanding of the molecular pathological processes and the clinical evaluation of new therapeutic approaches to heart disease in humans highlight a new role for ARA. The targets of ARA clearly have been widened to include aspects of cardiovascular remodelling in human heart disease. ARA appear to be a useful tool in counteracting the impact of RAAS activation, particularly if one considers the role of aldosterone in the progression of myocardial fibrosis, vascular remodelling and endothelial dysfunction.

The interplay between aldosterone and AngII at the target tissue level (myocardium, vascular smooth muscle cells and endothelium) is much more complex than we previously thought. Inhibition of ACE alone is not sufficient to block aldosterone production and aldosterone appears to potentiate the formation and actions of AngII even in human patients taking ACE inhibitors.

The molecular mechanisms of actions of ARA are beginning to be unravelled. Data which strongly suggest that ARA alter cardiovascular remodelling and hence provide therapeutic actions beyond their diuretic effects, seem to translate from humans and experimental animals to dogs.

**REFERENCES**


