Aldosterone synthase inhibition improves cardiovascular function and structure in rats with heart failure: a comparison with spironolactone

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Aims
Inhibition of aldosterone synthase, the key enzyme in aldosterone formation, could be an alternative strategy for mineralocorticoid-receptor antagonists in congestive heart failure (CHF), but its effect in CHF is unknown.

Methods and results
We compared, in rats with CHF, the effects of a 7 day and a 12 week treatment with the aldosterone synthase inhibitor FAD286 (4 mg kg⁻¹ day⁻¹) with those induced by spironolactone (80 mg kg⁻¹ day⁻¹). FAD286/spironolactone increased cardiac output without modifying arterial pressure. Long-term FAD286 and spironolactone reduced left ventricular (LV) end-diastolic pressure, LV relaxation constant, and LV dilatation, and these effects were more marked with FAD286, whereas both drugs reduced LV hypertrophy and collagen accumulation to the same extent. Long-term FAD286/spironolactone prevented CHF-related enhancement in LV ACE and reduction in LV ACE-2, but only FAD286 prevented the reduction in LV AT₂ receptors. FAD286, but not long-term spironolactone, reduced the CHF-related enhancements in LV reactive oxygen species, reduced-oxidized glutathione ratio, and aortic nicotinamide adenine dinucleotide phosphate oxidase activity. FAD286 normalized the CHF-induced impairment of endothelium-dependent vasodilatation.

Conclusion
In experimental CHF, FAD286 and spironolactone improve LV haemodynamics, remodelling, and function, but only FAD286 persistently normalizes LV ‘redox status’. These results suggest that aldosterone synthase inhibition is a potential therapeutic strategy for the treatment of CHF.

Keywords
Aldosterone • Heart failure • Spironolactone • Aldosterone synthase inhibition

Introduction
‘Aldosterone escape’ is observed with therapeutic ACE-inhibition in patients with congestive heart failure (CHF) and in experimental CHF models. Indeed, plasma levels of aldosterone are only transiently reduced after the introduction of ACE-inhibitor treatment and return towards pre-treatment levels subsequently, illustrating persistent aldosterone production.1–4 Furthermore, the beneficial effects in terms of morbi-mortality as well as quality of life of mineralocorticoid-receptor (MR) antagonists when added to ACE-inhibition among patients with severe CHF confirm the deleterious role of aldosterone in the progression of CHF.5,6 However, it must be stressed that plasma aldosterone concentrations increase even further during long-term treatment with MR antagonists,7 which might limit the magnitude of the MR antagonist’s protective effect. Moreover, now there is evidence that several of aldosterone-induced effects in the cardiovascular system are ‘insensitive’ to MR antagonists. Indeed, spironolactone does not prevent aldosterone-induced negative inotropic effect in human trabeculae,8 nor ischaemia-induced deterioration of myocardial contractile and metabolic functions,9 or the potentiation by aldosterone of angiotensin-II-induced vasoconstriction of coronary arteries.8
Thus, reduction in aldosterone levels through inhibition of aldosterone synthase, the key enzyme involved in aldosterone production, could be an alternative to MR antagonists for the treatment of CHF, since it will not only diminish effects mediated by receptors sensitive to MR antagonists but also those insensitive to MR antagonists. However, the effects of aldosterone synthase inhibition in CHF, and the mechanism(s) involved, are unknown.

As a primary endpoint, we compared, in a rat model of CHF, the long-term effects of the aldosterone synthase inhibitor FAD286 with those induced by the MR antagonist spironolactone on cardiac and vascular functions in CHF. Furthermore, several molecular mechanisms known to be involved in cardiovascular remodelling were evaluated as secondary endpoints.

Methods

This experimental investigation conforms to the Position of the American Heart Association on Research Animal Use, adopted by the AHA on 11 November 1984. All measurements were performed by observers blinded to prior results and treatment groups.

Animals and treatment

Induction of myocardial infarction

Myocardial infarction was produced in 11-week-old male Wistar rats by left coronary artery ligation over a 3 week period, as described previously. Briefly, rats were anesthetized (ketamine and xylazine; 60 and 5 mg/kg, respectively, IP), and after a thoracotomy, the proximal left coronary artery was occluded with a suture in order to induce myocardial infarction. Sham-operated rats, i.e. the last animal of each group, were subjected to the same protocol, except that the snare was not tied; 15 min after occlusion, the chest was closed and the animals were allowed to recover from anaesthesia.

Interpretation of the results obtained after long-term treatment does not allow separation of the direct, i.e. acute, effects of FAD286/spironolactone and indirect, i.e. long-term, effects induced by the improvement of haemodynamics and/or cardiac remodelling. In order to avoid this experimental bias, two separated protocols were performed.

Long-term treatment protocol

Eight days after ligation, 54 rats with myocardial infarction were randomized, in a 1:1:1 ratio (square Latin procedure) according to a randomization code generated before the start of the study, in three groups: untreated (n = 18), treated with the aldosterone synthase inhibitor FAD286 (4 mg kg\(^{-1}\) day\(^{-1}\); n = 18),\(^{11}\) or the MR antagonist spironolactone (80 mg kg\(^{-1}\) day\(^{-1}\); n = 18),\(^{12}\) while 12 untreated sham animals were used as control. The 14 infarcted animals that died after the surgical intervention but before randomization were excluded from the study.

Short-term treatment protocol

Eight days after ligation, 45 rats with myocardial infarction were randomized, as described earlier, in three groups: untreated (n = 15), treated with the aldosterone synthase inhibitor FAD286 (4 mg kg\(^{-1}\) day\(^{-1}\); n = 15), or the MR antagonist spironolactone (80 mg kg\(^{-1}\) day\(^{-1}\); n = 15), while untreated sham animals were used as control. As for the long-term treatment period, the 10 infarcted animals that died after the surgical intervention but before randomization were excluded from the protocol.

Cardiac and vascular functions

Left ventricular function

Transthoracic Doppler echocardiographic studies, using an echocardiographic system (HDI 5000, ATL, USA) equipped with an 8–5 MHz transducer, were performed in anaesthetized rats (Brietal™ 50 mg kg\(^{-1}\), IP) just before the start of the treatment (i.e. 7 days after the surgical procedure) and after 30 as well as 90 days of treatment, as described previously.\(^{10}\) In the short-term treatment protocol, echocardiographic measurements were made only at the end of the 7 day treatment. Briefly, a two-dimensional short-axis view of the left ventricle was obtained at the level of the papillary muscle in order to record M-mode tracings. LV diameters were measured by the American Society of Echocardiology leading-edge method from at least three consecutive cardiac cycles.\(^{13}\)

LV outflow velocity was measured by pulsed-wave Doppler, and cardiac output was calculated as \(CO = \text{aortic VTI} \times \left(\frac{\pi \times (\text{LV outflow diameter}/2)}{2}\right) \times \text{heart rate}\), where VTI is velocity–time integral.

Peripheral vascular function

After obtaining haemodynamic parameters, third-order mesenteric arteries (~1 mm in length; 150–175 μm inner diameter) were carefully dissected out for in vitro assessment of endothelium-dependent vasodilatation, as described previously.\(^{14,15}\) Vessels were then cannulated with glass micropipettes, pressurized to 90 cm H\(_2\)O intraluminal pressure, and bathed in physiological salt solution. The inner diameters of the vessels were measured using video microscopic techniques. After a 15 min equilibration period, the pressurized arteries were pre-constricted by addition of phenylephrine (3 × 10\(^{-5}\) mol/L), and the endothelial-dependent vasodilator response to increasing levels of intraluminal flow was determined as indicator of endothelial function.

Coronary vascular function

Coronary endothelial function was assessed as described previously.\(^{16}\) In brief, at the end of the experiments, the heart was removed and immediately placed in cold oxygenated KREBS buffer: A 1.5–2 mm long segment of the interseptal coronary artery was carefully dissected and mounted in a small vessel myograph (JP Trading; Aarhus, Denmark). Normalization procedure was performed after an equilibration period, as described previously.\(^{17}\) Segments with an internal diameter <170 μm were excluded to avoid mechanical endothelial injury and unspecific dysfunction. Concentration–response curves to acetylcholine (10\(^{-9}\)–3 × 10\(^{-5}\) mol/L) were obtained in serotonin-precontracted segments (10\(^{-5}\) mol/L). Serotonin was used because the coronary arteries in the rat do not express serotonin receptors on endothelial cells, and thus serotonin only induces smooth muscle cell contraction and no endothelium-dependent relaxations in these preparations. Endothelium-independent relaxation to increasing concentrations of sodium nitroprusside was also obtained in serotonin-pre-contracted arteries.
Left ventricular morphohistological assessment

After assessment of LV haemodynamics, atri and right as well as left ventricles were weighted separately, and a section of the left ventricle was immersed in fixative solution. After fixation, the sections were dehydrated and embedded in paraffin. From these sections, 5 μm thick histological slices were obtained and stained with Sirius Red.

Infarct size was determined as described previously. In brief, slices were placed under a video microscope and the endocardial and epicardial circumferences of the infarcted tissue of the left ventricle were determined using an image analysis software (Nacza; Microvision). Infarct size was calculated as the surface occupied by the endocardial and epicardial circumference of the left ventricle and expressed as a percentage.

LV collagen density in ‘viable’ part of the left ventricle was determined as described previously and expressed as the surface occupied by collagen divided by the surface of the image.

Matrix metalloproteinase MMP-2 gelatinolytic activity was measured in the non-infarcted left ventricle by gelatin zymography, as described previously. In brief, frozen LV tissue was crushed with a mortar and pestle at liquid nitrogen temperature and then homogenized by sonication in 50 mmol/L Tris–HCl (pH 7.4) containing 3.1 mmol/L sucrose, 1 mmol/L dithiothreitol, 10 μg/mL leupeptin, 10 μg/mL soyabean trypsin inhibitor, 2 μg/mL aprotinin, and 0.1% Triton X-100.

Samples were diluted with 0.5 mol/L Tris–HCl (pH 6.8), 10% sodium dodecylsulphate (SDS), 60% saccharose, and 1% bromophenol blue. Twenty micrograms of total proteins and gelatinase zymography standards (Chemicon International, USA) were loaded onto electrophoretic gel (10% SDS–PAGE) containing 1 mg/mL porcine gelatin (Sigma Aldrich). The gels were run at constant 200 V through the stacking phase and the separating phase, maintaining a running buffer at 4°C for 1 h, and then washed with 2.5% Triton X-100 twice for 15 min under agitation at room temperature. Gels were incubated overnight at 37°C in incubation buffer containing 10 mmol/L Tris base, 45 mmol/L Tris–HCl, 0.2 mmol/L NaCl, and 5 mmol/L CaCl₂. Gels were stained in 35% ethanol, 10% acetic acid, and 0.2% coomassie blue and were bleached in 35% ethanol, 10% acetic acid. Areas of MMP-2 digestion were visualized by negative staining, and quantification of proMMP-2 activity was performed by densitometry analysis with beta-digestion.

Aortic nicotinamide adenine dinucleotide phosphate oxidase activity

Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity was measured by superoxide-dependent lucigenin chemiluminescence, as described previously. Aortic tissues were minced and homogenized on ice in buffer containing 50 mmol/L monobasic potassium phosphate, pH 7.0, 250 mmol/L sucrose, and protease inhibitors (1 mg/mL aprotinin, 0.5 μg/mL leupeptin, and 87 μg/mL phenylmethylsulfonyl fluoride). Microsomal fractions were obtained from 100,000 g pellets. Microsomal fraction (30 μg of protein) was added to a glass scintillation vial in 50 mmol/L monobasic potassium phosphate (pH 7.0), containing 5 μmol/L lucigenin. Reaction was started by the addition of 500 μmol NADPH to the incubation medium as a substrate for O₂⁻ production. Luminescence was measured in a dark room with a scintillation counter (Wallac 1410). Measurements were integrated for a 1 min period and the cycle repeated three times. Background counts were determined by NADPH-free incubation. NADPH oxidase was expressed as cpm/min/30 μg proteins.

Left ventricular glutathione

Myocardial concentrations of total glutathione (oxidized glutathione GSSG + reduced glutathione GSH) and of the oxidized form (GSSG) were measured by glutathione reductase-5,5-dithiobis(DTNB) recycling assay as described previously.

Statistical analysis

All results are given as mean ± standard deviation. LV diastolic and systolic diameters as well as left haemodynamic parameters were assessed as a primary endpoint, whereas all other parameters, i.e. the molecular mechanisms, were assessed as secondary endpoints. Since no data were available on the possible effect of FAD286 on remodelling and haemodynamics, we made a simulation of the minimal sample size needed, and this for each parameter, to demonstrate a statistical significant (P > 0.05) variation of 10% vs. our historical data for each parameter obtained in untreated animals.

In order to evaluate the effect of CHF induced by coronary artery ligation, all parameters obtained in sham and untreated CHF animals were compared by Student’s t-test. In order to evaluate the effect of FAD286 or spironolactone, all parameters obtained in untreated and FAD286- and spironolactone-treated CHF animals were compared using a one-way ANOVA at each time interval, followed, in case of significance, by a two-sided
Tukey’s test for multiple comparisons. It must be stressed that three untreated, one FAD286-, and two spironolactone-treated CHF animals died during the last 60 days of the treatment period, inducing a bias in the statistical analysis at the 90 day time point. Differences between groups were considered significant at the level $P < 0.05$.

**Results**

**Effect of coronary artery ligation**

Seven and 90 days after ligation, mean arterial pressure and cardiac output were diminished in untreated CHF animals, whereas total peripheral resistance was increased (Table 1). Simultaneously, coronary ligation induced a decrease in LV end-systolic pressure as well as in LV $dP/dt_{max}$ or $dP/dt_{min}$, and an enhancement in LV end-diastolic pressure and LV relaxation constant $\tau$ (Figure 1). LV systolic and diastolic diameters were increased, whereas LV fractional shortening was decreased (data not shown). LV cavity dilation was associated with an increase in LV weight and a significant collagen accumulation in the viable part of the left ventricle (Table 2). Furthermore, coronary ligation induced a time-dependent modification of the expression of the ACE system. Although after 7 days only LV AT$_1$ receptor expression as well as urinary aldosterone levels were increased without any modification of ACE/ACE-2 and/or AT$_2$ receptor expression, all these parameters were either increased (ACE and AT$_1$ receptor expressions, urinary aldosterone levels) or decreased (ACE-2 and AT$_2$ receptor expressions) after 90 days (Table 3). However, enhanced myocardial oxidative stress was observed both 7 and 90 days after ligation (Figure 2).

Finally, after 90 days, coronary artery ligation provoked marked coronary and peripheral artery endothelium-dependent dysfunction associated with an enhanced oxidative stress (Figure 3).

**Cardiac haemodynamics and remodelling**

Compared with untreated CHF, FAD286 and spironolactone reduced mean arterial blood pressure after 90 days, but this effect reached statistical significance only for spironolactone, whereas none of the compounds modified blood pressure after 7 days of treatment (Table 1). Moreover, both FAD286 and spironolactone increased, to the same extent, cardiac output and reduced total peripheral resistance after 7 days of treatment, but these effects were more marked with FAD286 after 90 days (Table 1). After 7 days, neither FAD286 nor spironolactone significantly modified LV end-systolic and end-diastolic pressures, LV relaxation constant $\tau$, and LV $dP/dt_{max}$ or $dP/dt_{min}$, (Figure 1). After 90 days, FAD286 significantly reduced LV end-diastolic pressure as well as LV relaxation constant $\tau$ and increased LV $dP/dt_{max}$ and $dP/dt_{min}$, whereas spironolactone induced only a reduction in LV end-diastolic pressure and LV relaxation constant $\tau$ (Figure 1).

Concerning LV dilatation, FAD286 and spironolactone did not modify LV diastolic diameter after 7 days of treatment, but prevented further progression over time of LV cavity dilatation, illustrated by the diminished LV diastolic diameter after 30 and 90 days of treatment (Figure 4). Furthermore, both FAD286 and spironolactone reduced LV systolic diameter after 7 days and this effect persisted over time, as illustrated by the diminished LV systolic diameter after 30 and 90 days. LV fractional shortening was improved by both treatments after 7, 30, and 90 days (Figure 4). Although the effects of both drugs on LV diastolic and systolic diameters were similar during the first 30 days of treatment, the effects induced by FAD286 became significantly more marked after 90 days compared with those induced by spironolactone.

Concerning hypertrophy and extracellular matrix, both FAD286 and spironolactone reduced, to the same extent, LV weight and collagen density after 90 days of treatment, whereas none of the treatments modified the CHF-induced enhancement of MMP-2 activity. Although both treatments reduced pulmonary wet weight, this effect was more marked with FAD286 (Table 2).

**Urinary aldosterone levels**

Compared with sham-operated rats, urinary aldosterone concentrations were significantly increased in CHF animals, and FAD286 completely opposed the increase in aldosterone levels after 7 and 90 days. In contrast, spironolactone slightly increased aldosterone concentrations after 7 days of treatment and this increase was exaggerated over time since aldosterone concentrations were more than doubled after 90 days (Table 3).

![Table 1 Effects of FAD286 and spironolactone on blood pressure, cardiac output, and total peripheral resistance](image)
Left ventricular AT1 and AT2 receptors and ACE and ACE-2 expressions

After 7 days of treatment, FAD286, but not spironolactone, increased, without reaching statistical significance, ACE-2 and AT2 receptor protein levels, whereas ACE and AT1 receptor protein levels were not modified compared with untreated CHF rats. After 90 days of treatment, both FAD286 and spironolactone normalized the CHF-induced reduction in AT2 receptor protein levels (Table 3).

Left ventricular oxidative stress

Both FAD286 and spironolactone reduced LV ROS levels after 7 days, but the reduction in LV ROS persisted only with FAD286. Moreover, FAD286, but not spironolactone, normalized the CHF-induced reduction in myocardial GSH to GSSG ratio after 90 days (Figure 2).
Table 3 Effects of FAD 286 and spironolactone on myocardial ACE and angiotensin receptor expression

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<th>Sham</th>
<th>Chronic heart failure</th>
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<td>Untreated</td>
<td>FAD286</td>
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<td>LV ACE (AU)</td>
<td>D7: 2.49 ± 1.17</td>
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<td>2.43 ± 1.47</td>
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<td>D90: 6.82 ± 0.79</td>
<td>13.21 ± 0.77*</td>
<td>6.25 ± 0.20†</td>
<td>6.14 ± 0.82†</td>
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<td>LV ACE-2 (AU)</td>
<td>D7: 6.22 ± 2.09</td>
<td>5.04 ± 2.30</td>
<td>9.02 ± 2.31†</td>
<td>6.42 ± 1.85</td>
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<td>D90: 7.63 ± 2.36</td>
<td>0.83 ± 0.94*</td>
<td>6.31 ± 3.18†</td>
<td>7.00 ± 0.91†</td>
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<td>LV AT1 (AU)</td>
<td>D7: 1.21 ± 0.041</td>
<td>1.75 ± 0.19*</td>
<td>1.49 ± 0.28†</td>
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<td>D90: 0.81 ± 0.21</td>
<td>1.24 ± 0.16*</td>
<td>1.04 ± 0.39†</td>
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<td>LV AT2 (AU)</td>
<td>D7: 2.73 ± 1.33</td>
<td>1.90 ± 1.90</td>
<td>3.80 ± 1.80†</td>
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<td>D90: 2.48 ± 0.44</td>
<td>1.86 ± 0.85*</td>
<td>2.64 ± 0.80†</td>
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<td>Urinary aldosterone excretion (ng/24 h)</td>
<td>D7: 0.76 ± 0.16</td>
<td>0.94 ± 0.15*</td>
<td>0.75 ± 0.11†</td>
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<td>D90: 1.21 ± 0.27</td>
<td>6.58 ± 0.41*</td>
<td>0.95 ± 0.83†</td>
<td>13.39 ± 6.65†</td>
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Data are expressed as mean ± SD; LV, left ventricle; D7, 7 day treatment; D90, 90 day treatment; AT1, angiotensin-type 1 receptor; AT2, angiotensin-type 2 receptor; n = 10–17 per group.

*P < 0.05 vs. sham; †P < 0.05 vs. untreated chronic heart failure; ‡P < 0.05 vs. spironolactone-treated chronic heart failure.

Figure 2 Myocardial reactive oxygen species concentration after 7 and 90 days as well as myocardial GSH-to-GSSG ratio after 90 days determined in sham (white bars; n = 9) and congestive heart failure rats either untreated (black bars; n = 9–11) or FAD286-treated (up-hatched bars; n = 10–12) or spironolactone-treated (down-hatched bars; n = 9–12). Data are expressed as mean ± SD; *P < 0.05 vs. sham; †P < 0.05 vs. untreated heart failure; ‡P < 0.05 vs. spironolactone-treated chronic heart failure.

Vascular endothelium function and vascular nicotinamide adenine dinucleotide phosphate oxidase activity

Long-term treatment with the aldosterone synthase inhibitor FAD286 improved the CHF-induced impairment of flow-induced endothelium-dependent dilatation of mesenteric resistance arteries as well as acetylcholine-mediated endothelium-dependent relaxation of interseptal coronary arteries, whereas spironolactone was without any effect. Endothelium-independent dilatation/relaxation induced by sodium nitroprusside of both arteries was not modified (data not shown). At this time point, FAD286, but not spironolactone, normalized the CHF-enhanced aortic NADPH oxidase activity (Figure 3).

Discussion

Our results show that both long-term aldosterone synthase inhibition and MR antagonism improve cardiac haemodynamics and reduce LV dilation and hypertrophy as well as collagen accumulation, resulting in an improvement of cardiac systolic and diastolic functions. Furthermore, these long-term effects induced by aldosterone synthase inhibition are, in our experimental conditions, more marked than those induced by MR antagonism. This might be related, at least in part, to the fact that only aldosterone synthase inhibition improved vascular endothelial function and persistently normalized the CHF-induced enhancement in myocardial ROS production as well as the CHF-induced reduction in LV AT2 receptors/ACE-2 expression, although a causal relationship remains to be demonstrated.

The effects of FAD286 and spironolactone were evaluated in a rat model of CHF, which reproduces the major hallmarks of humans CHF. Indeed, marked progressive LV dilatation and depressed LV function were observed in placebo-treated CHF animals throughout the 90 day observation period. This was associated with an impairment of LV haemodynamics/function, development of LV hypertrophy, and collagen accumulation as well as neuro-humoral activation/enhanced oxidative stress, as already described in this model of CHF.18,21–24

Concerning LV remodelling and function, both long-term FAD286 and spironolactone associate ‘reversed’ LV remodelling and improved LV function. Simultaneously with a reduction in cardiac hypertrophy, both drugs reduced LV collagen accumulation, resulting from a reduced collagen synthesis while extracellular matrix turnover remains elevated. Indeed, collagen degradation remains elevated since MMP-2 gelatinase activity is not modified by any of the treatments, and this, together with the reduction in LV collagen density, suggests that, although collagen synthesis has not...
been determined, FAD286 and spironolactone reduce, directly or indirectly, collagen synthesis. However, it must be stressed that while the reductions in LV weight and collagen density induced by both treatments were of similar magnitude, the improvements in systolic and diastolic LV functions were, in our experimental conditions, significantly more marked after long-term FAD286. Indeed, the reductions in LV end-diastolic pressure, τ, and systolic and diastolic diameters as well as the increases in LV dP/dt_{max} and cardiac output were significantly more marked after long-term FAD286. Moreover, such a difference between FAD286 or spironolactone was not observed after short-term treatment, since after 7 or 30 days, the magnitude of the decrease in LV end-diastolic pressure/LV systolic diameter and the increase in cardiac output/fractional shortening induced by FAD286 or spironolactone were similar. This difference between the short- and long-term treatment with aldosterone synthase inhibition or MR antagonist might find its origin in the different adaptation of pro/anti-oxidant systems. Indeed, acute reduction in oxidative stress due to either scavenging of ROS by anti-oxidant administration or reducing concentration/production of ROS by pro-oxidant enzyme inhibition not only improves acutely LV systolic function but is on the long-term also associated with ‘reversed’ remodelling. Thus, the reduction in LV ROS observed in our study after short-term FAD286/spironolactone may contribute,

Figure 3 Flow-induced dilatation of third-order mesenteric resistance arteries, acetylcholine-induced vasorelaxation of interseptal coronary arteries, and aortic nicotinamide adenine dinucleotide phosphate oxidase activity determined in sham (open circles, white bars; n = 10) and congestive heart failure rats either untreated (solid circles, black bars; n = 12) or FAD286-treated (open triangles, up-hatched bars; n = 11) or spironolactone-treated (solid triangles, down-hatched bars; n = 9) at the end of the 90 day treatment. Data are expressed as mean ± SD; *P < 0.05 vs. sham; †P < 0.05 vs. untreated congestive heart failure.

Figure 4 Left ventricular diastolic and systolic diameters and left ventricular fractional shortening determined in congestive heart failure rats either untreated (circles; n = 15) or FAD286-treated (solid triangles; n = 15–17) or spironolactone-treated (open triangles; n = 15–17) during the 90 day treatment. Data are expressed as mean ± SD; †P < 0.05 vs. time-matched untreated congestive heart failure; ‡P < 0.05 vs. time-matched spironolactone-treated chronic heart failure.
at least in part, to the improvement of LV function as well as to the reduction in LV dilatation observed after chronic FAD286/spironolactone.

The fact that only FAD286 persistently reduced oxidative stress might be related to the involvement of both MR and non-MR-sensitive mechanism(s) in the enhancement of oxidative stress. First, activation of NADPH oxidase by aldosterone is only partially reduced by MR antagonists, whereas insuffi cient blockade MRs by spironolactone after 90 days, due to a progressive ‘up-regulation’ of MRs expression, as recently described, could explain the lack of long-term spironolactone treatment on oxidative stress in our study. Secondly, the normalization of the AT1/AT2 receptor ratio induced by FAD-286, but not by spironolactone, could lead to a reduction in myocardial and vascular oxidative stress. Indeed, AT2 receptors offset the AT1 receptor-mediated activation of NADPH oxidase by angiotensin II, whereas enhancement of oxidative stress, i.e. NADPH oxidase activity, induced by aldosterone is only completely blocked by co-administration of eplerenone together with AT1 receptors blockers. Finally, an increased neutralization of ROS by the reduction in LV dilatation observed after chronic FAD286/spironolactone treatment38,39 and the absence of any effect on endothelium-dependent vasodilatation after long-term spironolactone treatment in this and other studies.40,41 Besides AT2 receptor expression and improved NO bioavailability due to reduced NO scavenging by ROS, other mechanisms might be involved in the improvement of flow-induced vasodilatation by FAD286. Indeed, aldosterone reduces levels of an essential co-factor of NO synthase tetrabydrobipterin,42 which favours ‘NO–synthase uncoupling’, a situation in which NO–synthase produces ROS rather than NO.43 Finally, the improved physio-pathological status per se, and thus a reduced activity of vasoconstrictor systems, might account for the improvement of the vascular function observed with both aldosterone synthase inhibition and MR antagonism.

In conclusion, our results obtained in a rat model of CHF show that long-term FAD286 administration improves cardiac haemodynamics as well as function and prevents LV remodelling and suggest that aldosterone synthase inhibition could be a therapeutic strategy for the treatment of CHF. However, whether the more marked long-term effects of aldosterone-synthase inhibition, when compared with MR antagonism, on LV remodelling/haemodynamics will result in a more marked effect on survival, as well as the exact mechanism(s) involved, i.e. mineralocorticoid and/or non-MR-mediated, remains to be elucidated.

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