Fadrozole Reverses Cardiac Fibrosis in Spontaneously Hypertensive Heart Failure Rats: Discordant Enantioselectivity Versus Reduction of Plasma Aldosterone


Reversal of cardiac fibrosis is a major determinant of the salutary effects of mineralocorticoid receptor antagonists in heart failure. Recently, R-fadrozole was coined as an aldosterone biosynthesis inhibitor, offering an appealing alternative to mineralocorticoid receptor antagonists to block aldosterone action. The present study aimed to evaluate the effects of R- and S-fadrozole on plasma aldosterone and urinary aldosterone excretion rate and to compare their effectiveness vs. the mineralocorticoid receptor antagonist potassium canrenoate to reverse established cardiac fibrosis. Male lean spontaneously hypertensive heart failure (SHHF) rats (40 wk) were treated for 8 wk by sc infusions of low (0.24 mg/kg/d) or high (1.2 mg/kg/d) doses of R- or S-fadrozole or by potassium canrenoate via drinking water (7.5 mg/kg/d). At the high dose, plasma aldosterone levels were decreased similarly by R- and S-fadrozole, whereas urinary aldosterone excretion rate was reduced only by S-fadrozole. In contrast, whereas at the high dose, R-fadrozole effectively reversed preexistent left ventricular interstitial fibrosis by 50% (vs. 42% for canrenoate), S-fadrozole was devoid of an antifibrotic effect. The low doses of the fadrozole enantiomers did not change cardiac fibrosis or plasma aldosterone but similarly reduced urinary aldosterone excretion rate. In conclusion, R-fadrozole may possess considerable therapeutic merit because of its potent antifibrotic actions in the heart. However, the observed discordance between the aldosterone-lowering and antifibrotic effects of the fadrozole enantiomers raises some doubt about the mechanism by which R-fadrozole diminishes cardiac collagen and about the generality of the concept of lowering aldosterone levels to treat the diseased heart. (Endocrinology 149: 28–31, 2008)

Although isolated more than 50 yr ago, it took several decades before it was realized that the adrenocortical steroid hormone aldosterone plays a crucial role in pathological cardiovascular remodeling and chronic heart failure (1). The clinical relevance of aldosterone in chronic heart failure became evident in the CONSENSUS trial showing that the mortality of heart failure patients correlated with plasma aldosterone (2). Later, the RALES study demonstrated that the mineralocorticoid receptor antagonist spironolactone greatly reduced mortality and symptoms in patients with severe heart failure (3). This aroused renewed interest in aldosterone-related pharmacology, resulting among others in the launching of the mineralocorticoid receptor antagonist eplerenone, which proved to be salutary in post-myocardial infarction patients in the EPHESUS trial (4).

Mineralocorticoid receptor antagonists block aldosterone actions that may compromise heart function and structure and provoke arrhythmias, such as inducing excessive cardiac fibrosis, cardiovascular inflammation, and shifts in electrolyte and autonomic nervous balances (5). A particularly important determinant for the therapeutic benefit of mineralocorticoid receptor antagonists seems to be their ability to prevent and reverse cardiac fibrosis (5–7). Mineralocorticoid receptor antagonists improve clinical outcome only in heart failure patients with high cardiac collagen deposition, whereas patient prognosis correlates with the extent by which cardiac collagen levels are reduced (6, 7).

In view of the successes of mineralocorticoid receptor antagonists and the established role of aldosterone in various diseases, it is not surprising that various groups, including ours, started a quest for drug candidates that interfere with aldosterone action in a manner alternative to blocking its receptor, i.e., by decreasing aldosterone biosynthesis. However, the therapeutic potential of this concept still needs to be investigated.

Recently, FAD 286A, the R(+)-enantiomer of fadrozole (a racemate also known as fadrazole or CGS 16949 A), has been...
identified as an aldosterone biosynthesis inhibitor (8–10). It was shown to reduce aldosterone levels in rats (8, 9) and to (partially) prevent angiotensin II-induced mortality, cardiac remodeling, and renal damage in transgenic human renin- and angiotensinogen-overexpressing rats (8). However, no information exists on aldosterone synthesis inhibitors with respect to cardiac remodeling in animals without excessive renin-angiotensin-aldosterone system activation. Also, their ability to reverse preexisting cardiac fibrosis and their effectiveness compared with mineralocorticoid receptor antagonists have never been studied. Therefore, we evaluated the effects of R-fadrozole and its enantiomeric counterpart S-fadrozole on plasma aldosterone levels as well as urinary aldosterone excretion rate and compared their effectiveness vs. the mineralocorticoid receptor antagonist potassium canrenoate regarding their ability to reverse preexistent cardiac fibrosis in 40-wk-old lean spontaneously hypertensive heart failure (SHHF) rats. This hypertensive rat strain is characterized by extensive cardiac fibrosis and cardiac remodeling, which eventually results in heart failure (11).

Materials and Methods

Fadrozole was synthesized by SyMO-Chem (Eindhoven University of Technology) essentially as described (12) (route C). The fadrozole enantiomers (>97.5% purity) were separated by Chiral Technologies Europe (Illkirch, France).

Forty-week-old male lean SHHF rats (Charles River Laboratories, Boston, MA) were randomly assigned to six weight-matched groups (n = 5 each) to be treated with a sc infusion of 1) vehicle (4:6 vol/vol sodium phosphate buffer, pH 5), 2) R-fadrozole at a dose rate of 0.24 mg/kg (Illkirch, France), 3) R-fadrozole at 1.2 mg/kg, 4) S-fadrozole at 0.24 mg/kg, 5) S-fadrozole at 1.2 mg/kg, or 6) vehicle and potassium canrenoate via drinking water (7.5 mg/kg). For the infusions, osmotic minipumps (2 ml; Durect, Cupertino, CA) were implanted sc under isoflurane anesthesia. These pumps produce a constant flow of 2.5 μl/h for at least 4 wk. After 4 wk, pumps were exchanged under isoflurane anesthesia. After 8 wk of treatment, rats were killed under pentobarbitone anesthesia (60 mg/kg ip), and blood and organs were harvested for analyses and snap frozen. At d 50 of the treatment, 24-h urine was collected for steroid and electrolyte (flame photometry) analyses.

The experiments were performed according to institutional guidelines and approved by the local ethical committee for experimental animal use, conforming with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Mean arterial blood pressures were recorded at treatment end under isoflurane anesthesia. After 8 wk of treatment, rats were killed under pentobarbitone anesthesia (60 mg/kg ip), and blood and organs were harvested for analyses and snap frozen. At d 50 of the treatment, 24-h urine was collected for steroid and electrolyte (flame photometry) analyses.

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Mean arterial blood pressures were recorded at treatment end under pentobarbitone anesthesia, using a high-fidelity catheter tip micromanometer (Mikro-tip 1.4 F, SPR-671; Millar Instruments, Houston, TX) that was inserted into the right carotid artery.

Table 1. Characteristics of the treated animals

<table>
<thead>
<tr>
<th>Vehicle</th>
<th>Canrenoate</th>
<th>R-fad high</th>
<th>R-fad low</th>
<th>S-fad high</th>
<th>S-fad low</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW start (g)</td>
<td>444 ± 9.78</td>
<td>448 ± 6.18</td>
<td>444 ± 10.4</td>
<td>453 ± 13.0</td>
<td>445 ± 6.12</td>
</tr>
<tr>
<td>BW end (g)</td>
<td>449 ± 11.9</td>
<td>450 ± 6.45</td>
<td>477 ± 6.95</td>
<td>473 ± 16.9</td>
<td>450 ± 5.14</td>
</tr>
<tr>
<td>CI (mg/g)</td>
<td>3.95 ± 0.12</td>
<td>4.18 ± 0.17</td>
<td>3.94 ± 0.08</td>
<td>3.85 ± 0.06</td>
<td>4.01 ± 0.10</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>183 ± 4.4</td>
<td>203 ± 4.32</td>
<td>188 ± 12.4</td>
<td>195 ± 9.06</td>
<td>201 ± 5.33</td>
</tr>
<tr>
<td>Plasma Na (mm)</td>
<td>142 ± 1.14</td>
<td>146 ± 1.45</td>
<td>141 ± 2.25</td>
<td>145 ± 0.91</td>
<td>142 ± 2.02</td>
</tr>
<tr>
<td>Plasma K (mm)</td>
<td>5.31 ± 0.27</td>
<td>4.74 ± 0.27</td>
<td>4.50 ± 0.20</td>
<td>5.31 ± 0.31</td>
<td>4.98 ± 0.27</td>
</tr>
</tbody>
</table>

BW start and end, Body weights of the animals at treatment start and end (d 56); CI, cardiac index (ratio of heart and body weight); fad, fadrozole; MAP, mean arterial right carotid artery blood pressure, assessed under pentobarbitone anesthesia at treatment end. Plasma Na and K were measured at treatment end.

Results

The treatment groups showed no differences in their body weights, heart/body weight ratios, mean arterial blood pressures, or sodium or potassium levels in plasma (Table 1).

Plasma aldosterone levels (Fig. 1A) were reduced by 8 wk of treatment with the high dose of R-fadrozole, corroborating with findings by others (8, 9), and were also reduced to a similar extent by the high dose of S-fadrozole. Urinary aldosterone excretion rates were reduced to a similar extent by the low dose of R- and S-fadrozole (Fig. 1B). At the high dose, however, only for S-fadrozole was a significant reduction of urinary aldosterone excretion rate observed, resulting in a significantly lower urinary aldosterone excretion rate than for R-fadrozole. No significant changes in plasma corticosterone levels (Fig. 1C) or urinary corticosterone excretion rates (Fig. 1D) were seen. This indicates that the reduction of plasma aldosterone by fadrozole is due to inhibition of the final aldosterone biosynthesis steps, catalyzed by aldosterone synthase (CYP11B2), because corticosterone and aldosterone share common precursors in their biosynthesis (see e.g. Ref. 15).

Figure 2A shows that in untreated SHHF rats, LV cardiac collagen fractions were constant between 39 and 52 wk of age. After 8 wk of treatment (starting age, 40 wk), however, both canrenoate and the high dose of R-fadrozole (Fig. 2B) decreased LV collagen fractions by 42 and 50%, respectively, whereas other treatments had no effect. Hence, combining Fig. 2, A and B, shows that R-fadrozole reversed preexistent LV fibrosis at least equally efficiently as a mineralocorticoid receptor antagonist. The decrease in steady-state LV collagen levels is, however, not (solely) due to reduced procollagen gene transcription (Fig. 2C), because LV procollagen α1 type I and III mRNA levels are not reduced by any treatment; in fact, in high-dose R-fadrozole-treated animals, procollagen mRNAs tended to be increased (not significant in ANOVA). Moreover, assessment of mRNA expression levels of several
genes including those encoding reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase subunits gp91phox, p22phox, and p47phox, cycloxygenase-2 (COX-2), angiotensin converting enzyme (ACE), and osteopontin (OPN) in the LV of the heart; CYP11B1 and CYP11B2 in the adrenal; and renin in the kidney did not reveal any treatment effects (supplemental Table 2, published as supplemental data on The Endocrine Society’s Journals Online web site at http://endo.endojournals.org).

**Discussion**

The present study shows that in SHHF rats, preexistent LV fibrosis is effectively reduced by the high dose of the R-enantiomer of fadrozole. In contrast, at the high dose, both enantiomers reduce plasma aldosterone, whereas urinary aldosterone excretion rate was reduced only by S-fadrozole.

Cardiac fibrosis is a key phenomenon in pathological cardiac remodeling, resulting in increased myocardial stiffness and dispersion of electrical conductance, hence impaired cardiac function and increased risk for arrhythmias (16). Mineralocorticoid antagonists, even in combination with angiotensin converting enzyme inhibitors, reduce collagen synthesis and reverse existing cardiac fibrosis in heart failure patients (6, 7). Recently, R-fadrozole was coined as aldosterone synthetase inhibitor and shown to prevent angiotensin II-induced cardiac and renal damage (8) and to reduce aldosterone levels in rats (9, 10), but no information on the S-enantiomer was given. Here we show that the potential of R-fadrozole stretches beyond prevention in a situation of extensive renin-angiotensin-aldosterone system activation; R-fadrozole regresses existing cardiac fibrosis in SHHF rats at least as efficiently as mineralocorticoid receptor antagonists. This is an important observation, because the therapeutic outcome of heart failure patients is intimately linked to the extent of cardiac fibrosis (6, 7). Nevertheless, in the present study, neither R- nor S-fadrozole nor potassium canrenoate seemed to induce an apparent improvement of cardiac function when assessed (by similar methods as described in Ref. 14) by echocardiography and determination of basal and maximal LV $+\frac{dP}{dt}$ (contractility rate) or $-\frac{dP}{dt}$ (relaxation rate). This lack in functional improvement can probably be explained by the fact that SHHF rats at this age still display a normal cardiac function (11).

In the present study, both of the high doses of R- and S-fadrozole were shown to decrease plasma aldosterone in vivo in SHHF rats to similar extents. Because these aldosterone levels represent only a single time point, urinary aldosterone excretion rates were also determined as an indication of the 24-h aldosterone turnover. Although the observed treatment effects on the urinary aldosterone excretion rates are some-

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**Fig. 1.** A, Plasma aldosterone levels at treatment end (d 56). *, Significantly different from vehicle, $P < 0.05$ (ANOVA, LSD). B, Urinary aldosterone excretion at d 50 of treatment. *, Significantly different from vehicle, $P < 0.05$ (ANOVA, LSD); #, significantly different from R-fadrozole (R-fad) high, $P < 0.05$ (ANOVA, LSD). C, Plasma corticosterone levels at treatment end (d 56). D, Urinary corticosterone excretion at d 50 of treatment.

**Fig. 2.** A, Epicardial LV collagen tissue area fractions of untreated SHHF rats at ages between 39 and 52 wk. Each data point represents the collagen tissue fraction for one individual untreated SHHF rat, standardized for the mean observed value for 40-wk-old SHHF rats. B, The effect of treating SHHF rats for 8 wk with potassium canrenoate or two different infusion doses of R- or S-fadrozole (R- or S-fad) on epicardial LV collagen tissue area fractions. *, Significantly different from vehicle-treated animals, $P < 0.05$ (ANOVA, LSD). C, LV procollagen α1 type I and III mRNA levels relative to phosphoglycerate kinase-1 and standardized for vehicle-treated rats in SHHF rats after various treatments.
what complex, the combination of these data with the observed plasma aldosterone levels indicates that S-fadrozole reduced aldosterone production in the SHHF rats to (at least) a similar extent as R-fadrozole. Surprisingly, however, S-fadrozole was devoid of an antifibrotic effect, in contrast to R-fadrozole, which clearly decreased LV collagen. This is an important observation because it raises some doubt about the mechanism by which R-fadrozole lowers cardiac collagen and about the generality of the concept of lowering aldosterone levels to treat the diseased heart. Possibly, R-fadrozole reverses cardiac collagen by additional non-aldosterone-related, yet to be identified, mechanisms. Alternatively, S-fadrozole may exert additional, currently unknown, actions that mask the potential antifibrotic effect of lowering plasma aldosterone. In any case, this discrepancy indicates that decreasing plasma aldosterone levels as such is not sufficient to reduce cardiac fibrosis in every setting and that multiple factors determine the outcome.

These observations clearly underline the need for additional studies on the therapeutic potential of other compound classes of aldosterone synthase inhibitors, next to R-fadrozole; such studies are in progress in our laboratory. Nevertheless, the here described capability of R-fadrozole to regress cardiac fibrosis, combined with the cited findings by others, indicates that R-fadrozole is a pharmacological tool of considerable merit.

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References


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