Spironolactone Increases Nitric Oxide Bioactivity, Improves Endothelial Vasodilator Dysfunction, and Suppresses Vascular Angiotensin I/Angiotensin II Conversion in Patients With Chronic Heart Failure

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Background—The RALES study showed that spironolactone, added to conventional therapy for chronic heart failure, dramatically reduced mortality. We tested the hypothesis that this benefit was partially due to improvement in endothelial function and/or to amplified suppression of the vascular renin-angiotensin axis.

Methods and Results—We performed a randomized, placebo-controlled, double-blind crossover study on 10 patients with NYHA class II to III chronic heart failure on standard diuretic/ACE inhibitor therapy, comparing 50 mg/d spironolactone (1 month) versus placebo. Forearm vasculature endothelial function was assessed by bilateral forearm venous occlusion plethysmography using acetylcholine and N-monomethyl-L-arginine (L-NMMA), with sodium nitroprusside as a control vasodilator. Also, vascular ACE activity was assessed by use of angiotensin (Ang) I, with Ang II as a control vasoconstrictor. Spironolactone significantly increased the forearm blood flow response to acetylcholine (percentage change in forearm blood flow [mean 6 SEM], 177 6 29% versus 95 6 20%, spironolactone versus placebo; P,0.001), with an associated increase in vasoconstriction due to L-NMMA (235 6 6% versus 218 6 4%; P,0.05). The Ang I response was also significantly reduced with spironolactone (P,0.05), with Ang II responses unaltered.

Conclusions—Spironolactone improves endothelial dysfunction, increases NO bioactivity, and inhibits vascular Ang I/Ang II conversion in patients with heart failure, providing novel mechanisms for its beneficial effect on cardiovascular mortality. (Circulation. 2000;101:594-597.)

Key Words: spironolactone ■ endothelium ■ angiotensin ■ enzymes ■ heart failure ■ nitric oxide

Recently, the Randomized ALdactone Evaluation Study (RALES) trial was stopped prematurely because of a significant 30% survival advantage in patients receiving spironolactone in addition to standard therapy for chronic heart failure (CHF), including diuretics and ACE inhibition.1 Mechanisms contributing to this beneficial effect include blocking of the effect of aldosterone on potassium/magnesium loss, autonomic function, and myocardial fibrosis.2 Recent experimental observations suggest a whole new adverse effect of aldosterone, namely on the vasculature. The most intriguing data are in 1 tissue culture study in which aldosterone inhibited NO release.3 Experimental evidence also suggests that aldosterone amplifies tissue ACE and angiotensin (Ang) II responses.4-6 We therefore set out to see whether such novel vascular effects could be demonstrated for aldosterone in humans in vivo. We focused on patients with CHF so that our findings were directly relevant to the RALES study.
baseline forearm blood flow (FBF) was measured by forearm venous-occlusion plethysmography, as described before. Drugs were then infused (see below) into the study arm with a constant-rate infuser. FBFs were measured at each baseline and during the last 2 minutes of each drug infusion. Blood pressure was measured in the noninfused (control) arm at regular intervals throughout the study.

Drug Infusions
First, acetylcholine (Miochol, CIBAVision) was infused at 25, 50, and 100 nmol/min, each for 5 minutes. This was followed by sodium nitroprusside (David Bull Laboratories) at 4.2, 12.6, and 37.8 nmol/min, each for 5 minutes, and then Nω-monomethyl-L-arginine (L-NMMA; Clinalfa) at 1, 2, and 4 μmol/min for 5 minutes each. This was followed by Ang I (Clinalfa) at 64, 256, and 1024 pmol/min for 7 minutes each, and finally, Ang II (Clinalfa) was infused at 16, 64, and 256 pmol/min for 7 minutes each. Between the different drugs, the drug infusion set was flushed with saline for 20 to 30 minutes to allow sufficient time for the FBF to return to baseline values.

Acetylcholine is an endothelium-dependent vasodilator, and sodium nitroprusside is an endothelium-independent vasodilator. L-NMMA is a competitive NO synthase inhibitor. Ang I exerts vasoconstriction in this forearm model only through conversion in the vasculature to Ang II, and therefore any vasoconstriction elicited reflects vascular Ang I/Ang II conversion. Ang II BP, mm Hg

BP indicates blood pressure; SBP, systolic BP; DBP, diastolic BP; MAP, mean arterial pressure; ACh, acetylcholine; and SNP, sodium nitroprusside.

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Statistical Analysis
FBF values (expressed as mL · min⁻¹ · 100 mL forearm volume⁻¹) were converted to the ratio between the increase in blood flow in the infused arm and the blood flow in the control arm, expressed as percentage change in FBF from the baseline immediately preceding each drug administration (mean ± SEM).

Clinical characteristics between study visits were compared by Student’s paired t test, and FBF measurements for individual subjects were compared between treatments by 2-way ANOVA with repeated measures with correction for multiple comparisons for within-group effects. A value of P < 0.05 was considered significant.

Results
Subject Characteristics
A trend toward increased baseline FBF was observed in the spironolactone-treatment group compared with the placebo group, although this difference was statistically nonsignificant (2.9 ± 0.6 vs 3.8 ± 0.6 mL · min⁻¹ · 100 mL⁻¹, placebo versus spironolactone; P = 0.17). Baseline FBF values between the arms preceding each drug infusion were not different, indicative of adequate drug washout between each infusion phase.

There were also no significant changes in blood pressure either between or during each study day (Table 2). Urea,
creatinine, and plasma potassium assays are also shown in Table 2. There was no observed subjective or objective change in NYHA functional class between the 2 treatment periods.

Forearm Vascular Responses
Spironolactone produced a significant improvement in acetylcholine-mediated endothelium-dependent vasodilation ($P<0.001$ for difference between whole dose-response curves, Figure 1). Similarly, the vasoconstrictive response to L-NMMA was augmented with spironolactone therapy ($P<0.05$). In contrast, spironolactone had no effect on sodium nitroprusside responses ($P=0.84$).

A significant reduction in vasoconstriction was seen between spironolactone and placebo with regard to Ang I (see Figure 2, $P<0.05$). However, there was no significant difference between the treatment groups with regard to the forearm responses to the control vasoconstrictor Ang II ($P=0.89$).

Discussion
Our principal finding is that tonic NO bioactivity was significantly improved by spironolactone in CHF. Also, spironolactone attenuated Ang I– but not Ang II–mediated vasoconstriction, implying that spironolactone causes further inhibition of vascular Ang I/Ang II conversion even in the presence of chronic ACE inhibition. Blood pressure and NYHA functional class were unaffected by spironolactone, which implies that systemic effects per se were not responsible for our findings.

Two pieces of data support the concept that spironolactone improves endothelial function. First, aldosterone reduces NO bioactivity in tissue culture when stimulated by cytokines.\(^3\) Second, aldosterone correlates inversely with arterial compliance, which may be related in part to NO.\(^9\) Our study also suggests that aldosterone upregulates vascular conversion of Ang I into Ang II in CHF despite ACE inhibition. This is

![Graph](http://circ.ahajournals.org/)

Figure 1. FBF responses to acetylcholine, sodium nitroprusside, and L-NMMA after placebo (■) or spironolactone (▲) therapy. Values are mean±SEM. *$P<0.05$, **$P<0.001$ for differences between treatments.

Figure 2. FBF responses to Ang I and Ang II after placebo (■) or spironolactone (▲) therapy. Values are mean±SEM. *$P<0.05$ for differences between treatments.
intriguing, implying that feedback amplification occurs in the vascular renin-angiotensin system as opposed to the more usual feedback inhibition. Before our study, experimental evidence supported this mechanism, with the one exception that in tissue culture, aldosterone also amplified the response to Ang II, whereas we did not find this.4–6 One might think that the improved NO production would complicate interpretation of our Ang I/Ang II responses, but the fact that Ang II responses were unaltered by spironolactone suggests that a specific effect on vascular Ang I conversion was observed rather than a general effect on vasoconstrictor responses. Furthermore, we have previously shown that NO does not alter vascular Ang I conversion itself in vivo in humans.10

From this study, it is impossible to say whether the spironolactone-induced improvement in NO activity accounts for the whole improvement in endothelial vasodilator dysfunction or whether spironolactone has additional effects on other endothelium-derived vasodilating substances, such as prostacyclin or endothelium-derived hyperpolarizing factor (EDHF). It is unlikely but not impossible that some of the improvement observed in acetylcholine responses was mediated by prostaglandins, because all subjects were taking soluble aspirin chronically (mean treatment duration, 6.5 years) at a dose known to cause vascular cyclooxygenase inhibition in humans.11 EDHF is virtually impossible to study in our forearm model at present for various reasons. It is currently contentious whether this agent is potassium ion,12 free fatty acid/arachidonic acid metabolites, or other putative agents. Barium can block the passage of this potassium-ion/EDHF in some fashion, but barium may not be pharmacologically specific for EDHF alone in our model, and its potential toxicity may limit in vivo arterial investigation. Therefore, we state that endothelial vasodilator dysfunction was improved by spironolactone and that basal NO bioactivity was improved by spironolactone, but we cannot exclude additional effects on prostacyclin or EDHF.

In conclusion, we have shown that spironolactone improves vascular function by novel mechanisms that are likely to contribute importantly to the positive RALES results. Indeed, improving endothelial dysfunction has recently been suggested as a new therapeutic target in CHF.13 Spironolactone appears to be a treatment that not only achieves that target but also reduces total mortality in patients with heart failure.

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