Effects of spironolactone on endothelial function, vascular angiotensin converting enzyme activity, and other prognostic markers in patients with mild heart failure already taking optimal treatment

J E Macdonald, N Kennedy, A D Struthers

R ALES (randomised aldactone evaluation study) showed a 30% mortality reduction in severe congestive heart failure (CHF) with the addition of spironolactone. The recent EPHESUS (eplerenone postacute myocardial infarction heart failure efficacy and survival study) confirms this, although as in RALES, the EPHESUS patients had moderate to severe disease. These two studies now raise the question of whether spironolactone would produce the same benefit in patients with mild or asymptomatic CHF. Indeed the editorial accompanying the EPHESUS paper said that “trials are now needed to determine whether these drugs will be efficacious in less severe patients”. A second question is that despite EPHESUS there is still some uncertainty over whether the RALES benefit would occur in CHF in the presence of optimal treatment including β blockade. This uncertainty arises because of the adverse effects of triple neuroendocrine blockade in ValHeFT (valsartan heart failure trial), because the RALES trial predated the use of β blockers in CHF, and because the EPHESUS data arise from a different population—that is, patients immediately after a myocardial infarction. To answer both questions posed above, we have now conducted a study to determine whether spironolactone improves endothelial function and other prognostic markers in patients with NYHA class I–II CHF when added to optimal treatment including β blockade. This gives support to the hypothesis that the prognostic benefit seen in RALES (randomised aldactone evaluation study) and EPHESUS (epilrenene postacute myocardial infarction heart failure efficacy and survival study) may also occur in patients with milder CHF already taking standard optimal treatment.

The main mechanism now thought by most experts to explain why aldosterone blockade reduces mortality in CHF is that aldosterone blockade improves endothelial vascular function. This has been shown directly only in severe CHF so far, but in agreement is the observation that infused aldosterone causes endothelial dysfunction in normal humans and the observation that aldosterone blockade is antiatherosclerotic in animal studies. Other factors that also contribute to the overall benefit are the beneficial effects of aldosterone blockade on cardiac remodelling, vascular angiotensin converting enzyme (ACE), QT interval, and heart rate variability.

To address the question posed in the EPHESUS editorial, we have now conducted a study to determine whether spironolactone improves endothelial function and other prognostic markers in patients with NYHA class I–II CHF taking optimal treatment including β blockers (unless contraindicated). The final answer as to whether aldosterone blockade reduces mortality in this setting can be obtained.

Abbreviations: ACE, angiotensin converting enzyme; BNP, β-type natriuretic peptide; EPHESUS, eplerenone postacute myocardial infarction heart failure efficacy and survival study; FBFR, forearm blood flow ratio; HAD, hospital anxiety and depression; L-NMMA, N- monomethyl-L-arginine; MLWHF, Minnesota living with heart failure; NYHA, New York Heart Association; PIIINP, procollagen III N-terminal peptide; QTc, corrected QT interval; QTd, QT dispersion; RALES, randomised aldactone evaluation study; SDNN, standard deviation of all NN intervals; ValHeFT, valsartan heart failure trial
only by an expensive mega-trial. Funding for such a mega-trial is only likely to become available if positive results are seen in a study such as this, which measured important surrogates for cardiac death.

METHODS

Study population

Fifty seven patients with stable NYHA class I–II CHF were recruited after giving full, informed consent. At diagnosis, their CHF had all been at least class II but optimising their treatment had improved patients substantially into a stable, less symptomatic state. The Tayside committee on medical research ethics approved the study and it complies with the Declaration of Helsinki. Left ventricular systolic dysfunction on echocardiographic, angiographic, or nuclear imaging was mandatory and taken as a left ventricular ejection fraction <50% or fractional shortening <25% at some time in the recent past. All participants were taking ACE inhibitors or angiotensin II receptor blockers and their individually titrated β blocker dose (all for at least six months). All patients had been assessed for β blocker treatment so that the subgroup not taking β blockers either had a contraindication or were unable to tolerate them. In this way, the whole group was regarded as taking optimal treatment for them. Patients with serum sodium concentration <130 mmol/l, potassium >5.0 mmol/l, urea >10.0 mmol/l, or creatinine >221 mmol/l were excluded.

Randomisation and drug titration

Patients were randomly assigned in double blind, crossover fashion to spironolactone (three months) or placebo (three months). After three months, they were crossed over to the opposite treatment for three months. The starting dose of spironolactone was 25 mg/day. After two weeks this was doubled to the same maximum dose (50 mg) as in RALES. If side effects occurred or plasma urea and electrolytes became deranged (criteria above) the dose was halved. Patients unable to tolerate the minimum dose (12.5 mg/24 hours) were withdrawn.

Assessments after each treatment period

The assessments were comprehensive and hence we made them only at the end of each randomised treatment period and not at baseline. This is especially because assessment of endothelial function and vascular ACE require arterial puncture. Therefore, for ethical reasons, we limited assessments to the minimum necessary. Hence, we compared them directly, rather than subtracting baseline values from both, since baselines for each treatment would have then required four rather than two arterial puncture studies.15 16

Endothelial function

Studies were conducted in quiet, temperature controlled (24–26°C) surroundings at 9 am. Patients fasted from midnight. Prescribed medication was taken, except diuretics. After 20 minutes’ supine rest a 27 gauge steel needle (mounted on a 16 gauge epidural catheter) was positioned in the non-dominant brachial artery. After 10 minutes of saline infusion baseline forearm blood flow ratio (FBFR)17 was assessed by forearm venous occlusion plethysmography as described in detail elsewhere.4 Drugs were serially infused at increasing concentrations and FBFR was assessed during the final two minutes of each concentration. Between each infusion, the infusion set was flushed with saline until FBFR had returned to baseline levels.

Intra-arterial infusions

The endothelium dependent vasodilator acetylcholine (CIBAVision) was administered at 25, 50, and 100 nmol/min, each for five minutes. Then angiotensin I (Clinalfa) was infused at 64 256 and 1024 pmol/min for seven minutes each. In this model, vasoconstriction elicited by angiotensin I relies entirely on its conversion to angiotensin II. Next angiotensin II (Clinalfa) was infused at 16 and 64 pmol/min for seven minutes each. The competitive nitric oxide synthase inhibitor N-monomethyl-L-arginine (L-NMMA, Clinalfa) was then infused at 4 μmol/min for five minutes. After this, acetylcholine was co-infused with L-NMMA at 50 and 100 nmol/min, each for five minutes. Finally, the endothelium independent vasodilator sodium nitroprusside (David Bull Laboratories) was given at 37.8 nmol/min for five minutes.

Blood samples

Samples were collected between 11 am and 1 pm after 30 minutes’ supine rest and centrifuged. Plasma was stored at −70°C (peptides) or −20°C (other samples). Blinded operators performed assays in batches. Plasma β-type natriuretic peptide (BNP) samples were collected into EDTA and aprotonin and measured by radioimmunoassay (Bachem, St Helens, Merseyside, UK). Serum procollagen III N-terminal peptide (PIIINP) concentrations were assessed by an intact radioimmunoassay (Quidel, UK).

QT parameters

Standard 12 lead ECGs were recorded with a 4700A machine (Hewlett Packard, Palo Alto, California, USA). For each lead, the QT intervals of three consecutive non-ectopic complexes were measured and averaged. A digitising tablet (Calcomp, Anaheim, California, USA) with in-house software was used. The QT interval was measured from QRS onset to T wave cessation. If U waves were present, the end of the QT interval was taken at the nadir between the T and the U waves. The corrected QT (QTc) interval was calculated by Bazett’s method (QTc = QT/RR). QT dispersion (QTd) was calculated as the difference between the longest and shortest QT intervals. Measurements were performed by one blinded investigator (JEM). Any lead omitted on a patient’s first ECG was omitted on the second.

Heart rate variability

Twenty four hour ambulatory Holter monitoring was undertaken. Patients with pacemakers, atrial fibrillation, or intermittent bundle branch block were excluded. A two channel Tracker II analogue tape recorder (Reynolds Medical, Hertford, Hertfordshire, UK) was used with CM1 and CM5 leads. Data were processed with the Pathfinder 600 Analyser (Reynolds Medical) and manually edited19 for both time domain (mean RR interval, standard deviation of all NN intervals, SDNN, index of heart rate variability) and frequency domain parameters (total power, ultralow frequency, very low frequency, low frequency, and high frequency).15

Six minute walk

Subjects were asked to walk briskly between two markers spaced 20 m apart.

Questionnaire data

The Minnesota living with heart failure (MLWHF) and hospital anxiety and depression (HAD) questionnaires were administered.20 21

Statistical analysis

Forearm blood flow (ml/min/100 ml forearm volume) rates were converted to ratios between forearm blood flow in the
infused arm and that in the control arm. Percentage change in FBF from the baseline immediately preceding each infusion was calculated. FBF measurements were analysed by a general linear model with treatment and dose as fixed factors and patient number as a random factor. The interaction between dose and treatment was non-significant in all cases. Other data were compared between treatments by Student’s paired t tests. A probability value of p < 0.05 was considered significant. Results are expressed as mean (SEM) in the figures.

RESULTS
Fourteen patients did not complete the study, of whom eight were taking spironolactone (two had cramps and two had hyperkalaemia) and six were given placebo (one experienced lethargy and one died). Six developed intercurrent illness. Two patients withdrew without giving reasons. Forty three patients completed the study (mean (SD) spironolactone dose of 40.1 (2.02) mg).

Clinical characteristics
Weight and NYHA class were unchanged (table 1). Systolic and diastolic blood pressures were significantly lower among patients taking spironolactone (p = 0.004 and p = 0.005, respectively).

Endothelial function
Endothelial function and vascular ACE was measured in only 40 patients, since those taking warfarin (n = 3) obviously cannot undergo arterial puncture studies. Blood pressure was unchanged during studies (table 4). Spironolactone significantly improved acetylcholine induced endothelium dependent vasodilatation (p = 0.045) (fig 1), blunted angiotensin I induced vasoconstriction (p = 0.006) (fig 2), and augmented angiotensin II induced vasoconstriction (p = 0.044) (fig 3). Responses to sodium nitroprusside, L-NMMA, and co-infusion of L-NMMA and acetylcholine were unaltered (fig 4). Mean (SD) sodium nitroprusside responses were 340 (156)% with placebo and 369 (189)% with spironolactone (p = 0.399, t test).

Blood analysis results
The most relevant differences between placebo and spironolactone administration were that both plasma BNP (p = 0.039) and plasma PIIINP fell (p = 0.000) with spironolactone. The PIIINP fall was greater when placebo PIIINP was 3 mgl as in RALES.

QT parameters
QT indices were significantly reduced with spironolactone: QT (p = 0.009), QTc (p = 0.002), QTd (p = 0.037), and QTdc (p = 0.032) (table 2).

Heart rate variability data
Spironolactone significantly reduced ventricular extrasystoles (p = 0.030) (table 3). Couplets and triplets fell nonsignificantly. Heart rate variability measures did not improve, including early morning heart rate variability. The use of β blockers in this study appears to have negated the effect of spironolactone on heart rate variability seen in previous studies.14

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Baseline clinical data</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>67.5 (7.05)</td>
<td></td>
</tr>
<tr>
<td>Male/female</td>
<td>35/8</td>
<td></td>
</tr>
<tr>
<td>NYHA class I/II</td>
<td>15/28</td>
<td></td>
</tr>
<tr>
<td>Furosemide (mg)</td>
<td>33.0 (38.0)</td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>38 (88%)</td>
<td></td>
</tr>
<tr>
<td>Warfarin</td>
<td>3 (12%)</td>
<td></td>
</tr>
<tr>
<td>ACE inhibitor/ARB</td>
<td>36 (84%)/7 (16%)</td>
<td></td>
</tr>
<tr>
<td>Statin</td>
<td>25 (58%)</td>
<td></td>
</tr>
<tr>
<td>β Blocker</td>
<td>31 (72%)</td>
<td></td>
</tr>
<tr>
<td>Digoxin</td>
<td>4 (9%)</td>
<td></td>
</tr>
</tbody>
</table>

ACEI, angiotensin converting enzyme inhibitor; AF, atrial fibrillation; ARB, angiotensin receptor blocker; DBP, diastolic blood pressure; DCM, dilated cardiomyopathy; HF, heart failure; IHD, ischaemic heart disease; LVH, left ventricular hypertrophy; NIDDM, non-insulin dependent diabetes mellitus; NYHA, New York Heart Association; SBP, systolic blood pressure.
Six minute walk distance

Six minute walk distance was unaltered (table 2).

Questionnaire data

Total MLWHF questionnaire scores worsened significantly with spironolactone (p = 0.044) but there was no increase in physical dimension score (table 2). Total HAD and anxiety scores were also significantly worse after spironolactone (p = 0.035 and p = 0.021, respectively). These results were surprising but perhaps spironolactone produces subtle adverse effects that adversely influence quality of life. One possibility is the well known sexual side effects of spironolactone such as impotence and gynaecomastia.

Influence of β blockade

The improvements in PIIINP, BNP, endothelial function (acetylcholine), vascular ACE (angiotensin I vasoconstriction), and QTc were also seen when the analysis was limited to the smaller number of patients who were taking β blockers (p = 0.000, p = 0.016, p = 0.076, p = 0.025, and p = 0.002, respectively). This shows that spironolactone still has beneficial effects even when prescribed as a third neuroendocrine blocking agent.

DISCUSSION

Our main finding is that spironolactone improved our primary end point of endothelial function in patients with class I–II CHF taking optimal treatment. There are now seven separate studies showing that endothelial dysfunction is associated with future cardiovascular events. In addition, the two treatments that are known to improve prognosis in CHF also improve endothelial function—that is, ACE inhibitors and spironolactone. Thus, spontaneous abnormalities in endothelial function in general and treatment induced changes in endothelial function in CHF have so far always predicted prognosis. Indeed, a recent editorial in *Circulation* called endothelial function a “barometer of vascular health representing an orchestrated response to the many processes which contribute to atherosclerosis development and progression.”

Angiotensin I induced vasoconstriction was blunted by spironolactone, implying reduced angiotensin I to angiotensin II conversion, and above that achieved with chronic ACE inhibitor treatment, as previously shown in patients with more severe CHF. This also agrees with data showing that aldosterone increases ACE mRNA 23-fold in tissue culture and with other experimental data. In our study this was despite spironolactone significantly increasing angiotensin II conversion over and above that achieved with chronic ACE inhibitor treatment, as previously shown in patients with more severe CHF.

In this study, we used BNP as an index of intracardiac pressure/volume and left ventricular remodelling. We also used PIIINP as an index of ventricular collagen turnover. Use of these plasma markers in this way is well established. Furthermore, both such markers are prognostic markers in CHF. Consistent reductions in both suggest improved left ventricular remodelling. Our intention was to confirm this with echocardiography but good images were obtained for only 21 patients because smoking and obesity were.
common in our cohort. Even in these 21 patients, we did find significant confirmatory reductions in left ventricular internal diameter (5.40 [0.18] cm to 5.06 [0.19] cm, p = 0.006) and left ventricular mass index (122 [8] g/m² to 112 [7] g/m²; p = 0.036), but these echocardiographic reductions were unreliable because they refer to only a subset of patients. Nevertheless, changes in BNP and PIIINP are so well linked to prognosis and remodelling in CHF that favourable changes in both of them are a further promising sign. As to left ventricular remodelling, the fact that we measured so many things could be seen as a weakness, as some positive effects may then be seen by chance. This is unlikely, since all of the positive effects we found have been seen before in patients with more severe CHF, which makes it unlikely that these effects occurred purely by chance in these mild to asymptomatic patients. Spironolactone did appear to worsen significantly one quality of life score and depression scores. Unlike the positive effects, such effects have not been seen before. These adverse scores may relate to adverse side effects of the drug, which were too subtle to lead to their being recognised as adverse drug effects as such. In addition, these patients had such mild CHF that it may have been difficult to improve their quality of life when it was fairly normal to start with. Obviously this is an important issue needing further study, since this finding may detract importantly from the beneficial effects seen in vascular and other prognostic markers.

**Conclusion**

Spironolactone improves endothelial nitric oxide bioactivity, vascular ACE activity, QT interval length, and other prognostic markers (BNP, PIIINP) when added to optimally treated patients with class I–II CHF. Although such effects have been seen before in moderate to severe CHF, the key point here is that we found them the first time ever in patients with mild CHF already taking optimal treatment. Most pertinent is that this is the first study to show beneficial effects in mild to asymptomatic CHF on the main mechanism that is thought to underlie its benefits—that is, endothelial function. A mega-trial should now be undertaken to establish whether these improvements in surrogate markers translate into a significant prognostic benefit and in particular to see whether any putative benefit outweighs the not inconsiderable and unpredictable risks of hyperkalaemia when spironolactone and ACE inhibitors are given together in certain patients with CHF. Such mega-trials are expensive that underpinning data such as these are essential before any funding body is going to be persuaded to release the necessary funds.

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