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RESEARCH LETTER

No influence of spironolactone on plasma concentrations of angiotensin-converting enzyme 2: Findings from the HOMAGE randomized trial

Pas d'influence de la spironolactone sur les concentrations plasmatiques de l'enzyme de conversion de l'angiotensine 2 : résultats de l'essai randomisé HOMAGE

Keywords SARS-CoV-2; COVID-19; Spironolactone
Mots clés SARS-CoV-2 ; COVID-19 ; Spironolactone

Background

Angiotensin-converting enzyme 2 (ACE2) is a functional receptor for SARS-CoV-2, and its expression increases with age, in men, and in people with chronic comorbid conditions who are most vulnerable to and have a worse prognosis from COVID-19 [1—3]. Speculation exists that inhibitors of the renin-angiotensin-aldosterone system, particularly angiotensin-converting enzyme (ACE) inhibitors, angiotensin-receptor blockers (ARB) and mineralocorticoid receptor antagonists (MRA) could up-regulate ACE2 expression, thus potentially increasing the availability of receptors for SARS-CoV-2 entry into host cells [4—6]. On the other hand, the soluble circulating form of ACE2 may act as a competitive blocker of SARS-CoV-2 by preventing binding of the viral particle to the surface-bound, full-length ACE2 [1]. In observational studies, the use of ACE inhibitors and ARB has not been associated with increased risk of SARS-CoV-2 infection or severe COVID-19 [7—9]. It has also been hypothesized that ARB may benefit patients with COVID-19. The rationale is that high concentrations of angiotensin II in the lung interstitium can aggravate inflammation that may culminate in respiratory failure, and that ARB could block the effects of angiotensin II [10]. The BRACE CORONA trial, in which patients hospitalized with mild-to-moderate COVID-19 who were taking ACE inhibitors or ARB before hospital admission were randomized to discontinuing or continuing ACE inhibitors or ARB, found no significant difference in the mean number of days alive and out of hospital for those assigned to discontinue versus continue these medications [11]. The REPLACE COVID trial did not find any difference in outcomes comparing strategies of discontinuing versus continuing ACE inhibitors and ARB in patients with COVID-19 [12]. Other ongoing randomized controlled trials (RCT) are also prospectively investigating whether ACE inhibitors or ARB should be given or withheld in patients at risk of or with SARS-CoV-2 infection (e.g. ClinicalTrials.gov Identifiers: NCT04353596, NCT04508985, NCT04355936, NCT04356495). Little information on the effects of MRA on plasma ACE2 levels exists, but some have speculated that spironolactone may reduce the inflammatory pathways and fibrosis complicating SARS-CoV-2 infection [7,13] and RCT are currently testing this hypothesis (NCT04424134).

The HOMAGE (Heart OMics in AGEing) clinical trial randomized patients at risk of developing heart failure to spironolactone in addition to usual care or usual care alone, for up to 9 months. We measured plasma concentrations of ACE2 at baseline and during follow-up, which provided an opportunity to investigate the effects of spironolactone on plasma concentrations of ACE2.

Methods

The HOMAGE trial (NCT02556450) had a prospective, randomized, open-label, blinded-endpoint, multicentre design, in which people at increased risk of developing heart failure were randomly assigned to receive either spironolactone in addition to standard care or usual care alone (''control''), for up to 9 months. The rationale, trial design and main results have been published [14,15]. The study was approved by relevant ethics committees and regulatory bodies. All participants provided written informed consent. The main inclusion criteria were age $\geq$ 60 years, increased risk of cardiovascular events (defined as the presence of at least two of the following risk factors: diabetes, hypertension, microalbuminuria or an abnormal electrocardiogram) for coronary artery disease, and ventricular dysfunction as evidenced by an NT-pro-B-type natriuretic peptide (BNP) concentration between 125 and 1000 ng/L or BNP between 35 and 280 ng/L. The main exclusion criteria were glomerular filtration rate (eGFR) < 30 mL/min/1.73 m^2, serum potassium > 5.0 mmol/L, atrial fibrillation or flutter, left ventricular ejection fraction < 45%, heart failure or treatment with loop diuretics.

Plasma concentrations of ACE2 were measured from samples taken at baseline, 1 month and at the final trial visit. Assays were done at the TATAA biocentre (Göteborg, Sweden), using high-throughput Olink Proseek® Multiplex technology cardiovascular II panel, as part of a

Abbreviations: ACE, angiotensin-converting enzyme; ARB, angiotensin-receptor blocker; HOMAGE, Heart OMics in AGEing; MRA, mineralocorticoid receptor antagonist; NPX, normalized protein expression.

Table 1 Baseline characteristics of the population by tertile of plasma ACE2 concentration (NPX).

Characteristics	Plasma ACE2 concentration at baseline			*P*
	Tertile 1 (n = 172)	Tertile 2 (n = 172)	Tertile 3 (n = 172)	
ACE2 (NPX)	3.0 ± 0.2	3.5 ± 0.1	4.3 ± 0.5	—
Age (years)	73 (69, 79)	73 (68, 79)	73 (69, 77)	0.40
Men	101 (59)	136 (79)	147 (86)	< 0.001
Coronary artery disease	111 (65)	132 (77)	128 (74)	0.028
Diabetes mellitus	65 (38)	61 (36)	82 (48)	0.050
Hypertension	139 (81)	132 (77)	134 (78)	0.64
Stroke	8 (5)	6 (34)	14 (8)	0.14
Beta-blocker	112 (65)	127 (74)	119 (69)	0.21
ACE inhibitor	96 (56)	81 (47)	94 (55)	0.21
Angiotensin-receptor blocker	44 (26)	47 (27)	50 (29)	0.77
Calcium-channel blocker	38 (22)	37 (22)	34 (20)	0.86
Thiazide	31 (18)	27 (16)	28 (16)	0.83
Statin	134 (78)	137 (80)	154 (90)	0.010
Body mass index (kg/m^2)	27 (24, 30)	28 (25, 31)	29 (27, 32)	< 0.001
Systolic blood pressure (mmHg)	140 (126, 157)	140 (126, 155)	140 (130, 153)	0.84
Diastolic blood pressure (mmHg)	77 (71, 84)	78 (71, 86)	79 (71, 85)	0.45
Heart rate (bpm)	62 (55, 68)	61 (54, 67)	60 (55, 68)	0.83
Left ventricular ejection fraction (%)	63 (59, 67)	62 (57, 66)	63 (58, 67)	0.42
Left ventricular mass (BSA indexed) (g/m^2)	92 (79, 110)	93 (81, 115)	97 (84, 112)	0.19
Left atrial volume (BSA indexed) (mL/m^2)	30 (26, 36)	31 (26, 36)	30 (25, 36)	0.77
E/e' ratio	9 (7, 11)	9 (7, 12)	9 (8, 12)	0.35
E/A ratio	0.9 ± 0.3	0.9 ± 0.3	0.9 ± 0.3	0.26
Tricuspid annular plane systolic excursion (mm)	23 (17, 27)	22 (18, 26)	22 (16, 26)	0.45
Mitral annular plane systolic excursion (mm)	16 (14, 18)	15 (13, 17)	15 (13, 18)	0.16
eGFR ($mL/min/1.73\,m^2$)	72 (60, 84)	73 (63, 83)	72 (61, 89)	0.75
Haemoglobin (g/dL)	13.9 (12.9, 14.7)	14.1 (13.2, 15.1)	14.1 (13.3, 14.9)	0.11
Sodium (mmol/L)	139 (138, 141)	140 (138, 142)	139 (138, 141)	0.40
Potassium (mmol/L)	4.3 (4.1, 4.5)	4.3 (4.1, 4.6)	4.4 (4.1, 4.6)	0.40
Urea (mmol/L)	9 (6, 15)	9 (6, 14)	9 (6, 13)	0.77
NT-pro-B-type natriuretic peptide (pg/mL)	211 (138, 363)	227 (135, 368)	202 (128, 327)	0.39
Galectin-3 (ng/mL)	16 (14, 20)	16 (13, 19)	16 (13, 20)	0.68
Procollagen type I carboxy-terminal propeptide (ng/mL)	78 (65, 95)	80 (64, 98)	81 (70, 100)	0.38
Procollagen type III N-terminal propeptide (ng/mL)	3.7 (3.0, 4.6)	3.9 (3.0, 5.0)	4.2 (3.3, 5.3)	0.004
Carboxy-terminal telopeptide of type I collagen (ng/mL)	3.7 (2.9, 4.9)	3.7 (2.8, 4.9)	3.8 (2.8, 5.0)	0.99

ACE: angiotensin-converting enzyme; BSA: body surface area; eGFR, estimated glomerular filtration rate; NPX: normalized protein expression. Data are expressed as number (%), mean ± standard deviation or median (25th to 75th percentile).

multiomics programme investigating mechanisms of progression to heart failure. Each kit uses a proximity extension assay technology, providing log_2 normalized protein expression (NPX) values with relative rather than ponderal or molecular concentrations (https://www.olink.com/).

The primary outcome of interest was the change in plasma concentrations of ACE2 from baseline to the final visit using analysis of covariance comparing the difference of changes between the control and spironolactone groups in the regression model. A linear regression model was fitted, with the change in concentration (last visit − baseline) as the outcome variable, a binary variable to indicate the treatment group (control/spironolactone) and the baseline ACE2 value (NPX units) as covariates. The treatment effect was the coefficient that resulted from the comparison of spironolactone and control in the regression model. Similar

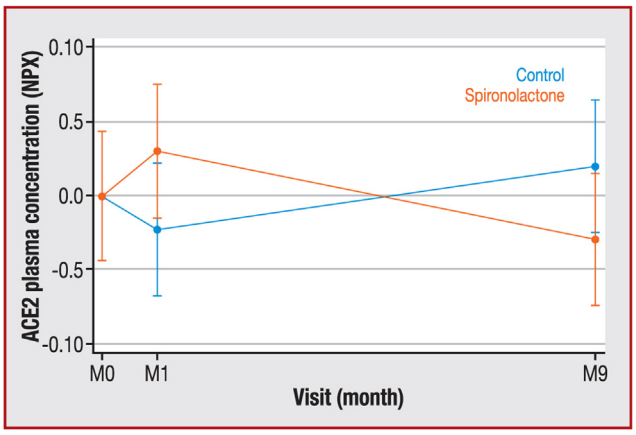

Figure 1. Change in circulating angiotensin-converting enzyme 2 (ACE2) concentration in patients randomized to spironolactone plus usual care or usual care alone: spironolactone did not significantly change ACE2 levels ($P = 0.083$ at month 1 and 0.44 at month 9).

analyses were performed for changes between baseline and 1 month. Statistical analyses were performed using Stata® (version 16, StataCorp LP).

Results

A total of 527 patients were randomized (265 to spironolactone and 262 to control). Median (25th to 75th percentile) follow-up was 8.9 (6.0, 9.2) months. The characteristics of the patients by ACE2 tertile are detailed in Table 1. Plasma concentrations of ACE2 were significantly ($P < 0.05$) associated with male sex ($\beta = 0.95$), diabetes ($\beta = 0.37$), higher body mass index ($\beta = 1.09$) and higher concentrations of procollagen type III N-terminal propeptide ($\beta = 0.61$), but not with age or coronary artery disease. Plasma concentrations of ACE2 were similar for the overall population at baseline, 1 and 9 months (3.6 ± 0.7 log2 at all time-points); changes from baseline did not differ for those assigned to spironolactone or control (1 month 0.06 [−0.01 to 0.13]; 9 months −0.03 [−0.11 to 0.05]) (Fig. 1). Stratification by risk subgroups of sex (women vs. men), diabetes (presence vs. absence) and body mass index (< 25 vs. 25 − 30 vs. > 30 kg/m^2) did not influence the neutrality of spironolactone effect (interaction $P > 0.1$ for all).

Discussion

Administration of spironolactone did not substantially change plasma ACE2 concentrations. These data do not support stopping or withholding MRA in patients with SARS-CoV-2 infection who have an indication for these drugs. Moreover, in agreement with other reports [3,7,16], we found that plasma concentrations of ACE2 were higher in men with diabetes and higher body mass index and, therefore, presumably with a higher risk of cardiovascular events independent of COVID-19. Whether the effects of MRA on inflammatory pathways and fibrosis have a favourable effect on the long-term outcome of patients with COVID-19 should also be considered. Trials formally testing safety and efficacy are already underway and more are planned.

Some limitations should be acknowledged. Our results may only apply to populations with characteristics similar to those in the HOMAGE trial. Our patients did not have SARS-CoV-2 infection and we cannot establish any association between the use of spironolactone and the risk or course of COVID-19. We measured only plasma ACE2 and do not know how well this reflects membrane-bound ACE2.

In conclusion, spironolactone did not substantially change plasma concentrations of ACE2 in this randomized trial, somewhat allaying concerns that MRA may increase the risk of SARS-CoV-2 infection or worsen its outcome. These findings do not support withholding MRA, if clinically indicated, in the context of SARS-CoV-2 infection. The results of randomized trials are awaited.

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Disclosure of interest

The authors declare that they have no competing interest.

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