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

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Abstract

Angiotensin-converting enzyme 2 (ACE2) is a functional receptor for SARS-CoV-2. Mineralocorticoid receptor antagonists (MRAs) might increase expression of ACE2. We aim to determine whether spironolactone changes plasma concentrations of ACE2 in the HOMAGE (Heart OMics in AGEing) trial. Patients were randomized to either spironolactone or usual care. Male sex, CAD, diabetes, and higher BMI were associated with higher plasma ACE2. Plasma concentrations of ACE2 were similar during follow-up (3.6±0.7 log2 at all time-points) and changes from baseline were not different between spironolactone and control. These findings support the safety of MRAs in the context of SARS-CoV-2.

Trial registration: ClinicalTrials.gov Identifier: NCT02556450

Key-words: SARS-CoV-2, COVID-19, spironolactone.

Introduction

Angiotensin-converting enzyme 2 (ACE2) is a functional receptor for SARS-CoV-2, and its expression increases with age, in men, and 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 (RAASi), particularly angiotensin-converting–enzyme inhibitors (ACEi), angiotensin-receptor blockers (ARBs), and mineralocorticoid receptor antagonists (MRAs) 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 the binding of the viral particle to the surface-bound, full-length ACE2.[1] In observational studies, the use of ACEi and ARBs has neither been associated with increased risk of SARS-CoV-2 infection nor severe COVID-19[7-9]. It has also been hypothesized that ARBs might 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 ARBs could block the effects of angiotensin II[10]. The BRACE CORONA trial that randomized patients hospitalized with mild to moderate COVID-19 who were taking ACEIs or ARBs before hospital admission to either discontinuing vs. continuing ACEi or ARBs, found no significant difference in the mean number of days alive and out of the hospital for those assigned to discontinue vs. continue these medications[11]. The REPLACE COIVD trial also did not find any difference in outcomes comparing strategies of discontinuing vs. continuing ACEi and ARBs in patients with COVID-19[12]. Other ongoing randomized controlled trials (RCTs) are also prospectively investigating whether ACEi and ARBs should be given or withheld in patients at risk or with SARS-CoV-2 infection (e.g.,

ClinicalTrials.gov Identifiers: NCT04353596, NCT04508985, NCT04355936, NCT04356495). Little information on the effects of MRAs on plasma ACE2 levels exists but some have speculated that spironolactone might reduce the inflammatory pathways and fibrosis complicating SARS-CoV-2 infection[7, 13] and RCTs are currently testing this hypothesis (NCT04424134).

The HOMAGE (Heart OMics in AGEing) clinical trial, randomized patients at risk of developing heart failure (HF) either to spironolactone or not, in addition to care, for up to nine months. We measured plasma concentrations of ACE2 at baseline and during follow-up, which provides an opportunity to investigate the effects of spironolactone on plasma concentrations of ACE2.

Methods

The HOMAGE trial was a prospective, randomized, open-label, blinded-endpoint (PROBE), multicentre design, in which people at increased risk of developing HF were randomly assigned to receive either spironolactone or not ("control") in addition to standard care (ClinicalTrials.gov Identifier: NCT02556450) for up to nine months. The rationale, trial design and main results have been published[14, 15]. The study was approved by all relevant ethics committees and regulatory bodies. All participants provided written informed consent prior to study-specific procedures. The main inclusion criteria were age 60 years or older, increased risk of cardiovascular events defined as the presence of or at least two of the following risk factors (diabetes, hypertension, microalbuminuria or an abnormal ECG) for coronary artery disease, and ventricular dysfunction as evidenced by a NT-pro BNP between 125 and 1,000 ng/L or BNP between 35 and 280 ng/L. The main exclusion criteria were glomerular filtration rate (eGFR) <30 mL/minute/1.73m2, serum potassium >5.0 mmol/L,

atrial fibrillation/flutter, left ventricular ejection fraction <45%, a diagnosis of HF or treatment with loop diuretics.

Plasma concentrations of ACE2 were measured from samples taken at baseline, one month and at the final trial visit. Assays were done at the TATAA-biocenter using the high-throughput Olink Proseek® Multiplex technology cardiovascular II panel, as part of a multi-omics programme investigating mechanisms of progression to HF. Each kit uses a proximity extension assay (PEA) 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 (ANCOVA) 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 analyses were performed for changes between baseline and one 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 standard of care). The median ($percentile_{25-75}$) follow-up time was 8.9 (6.0-9.2) months. The characteristics of the patients by ACE2 tertiles are depicted in *Table 1*. Plasma

concentrations of ACE2 were significantly ($p<0.05$) associated with male sex (β =0.95), diabetes (β =0.37), higher body mass index (β =1.09) and higher concentrations of procollagen type III N-terminal propeptide (β =0.61), but not with age nor coronary artery disease. Plasma concentrations of ACE2 were similar for the overall population at baseline, one and nine months (3.6±0.7 NPX at all time-points); changes from baseline were not different for those assigned to spironolactone or control (one month +0.06 (-0.01 to +0.13); nine months -0.03 (-0.11 to +0.05). *Figure 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/m2) did not influence the neutrality of spironolactone effect (interaction P >0.1 for all).

Discussion

Administration of spironolactone did not substantially change plasma ACE2. These data do not support stopping or withholding MRAs in patients with SARS-CoV-2 infection who have an indication for these agents. 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, independently of COVID-19. Whether the effects of MRA on inflammatory pathways and fibrosis might have a favourable effect on the long-term outcome of 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 HOMAGE. 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 might increase the risk of SARS-CoV-2 infection or worsen its outcome. These findings do not support withholding MRAs, if clinically indicated, in the context of SARS-CoV-2 infection. The results of randomized trials are awaited.

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Compliance with Ethical Standards

The authors have no conflicts of interest to report regarding the content of this manuscript.

All authors provided written informed consent to participate in the study.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Ethics approval was obtained at each participating site.

References

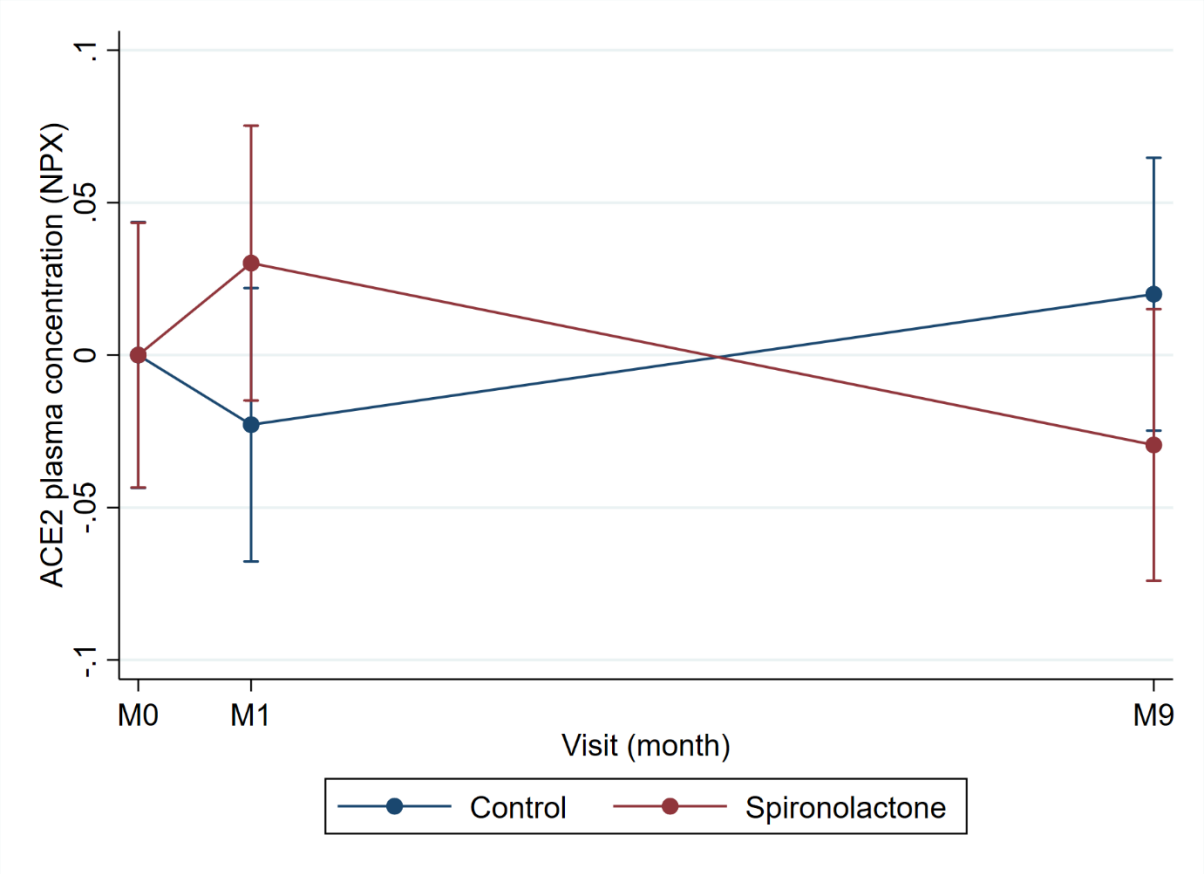
1. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature*. Nov 27 2003;426(6965):450-4. doi:10.1038/nature02145
2. Onder G, Rezza G, Brusaferro S. Case-Fatality Rate and Characteristics of Patients Dying in Relation to COVID-19 in Italy. *Jama*. May 12 2020;323(18):1775-1776. doi:10.1001/jama.2020.4683
3. Narula S, Yusuf S, Chong M, et al. Plasma ACE2 and risk of death or cardiometabolic diseases: a case-cohort analysis. *Lancet*. Oct 3 2020;396(10256):968-976. doi:10.1016/s0140-6736(20)31964-4
4. Diaz JH. Hypothesis: angiotensin-converting enzyme inhibitors and angiotensin receptor blockers may increase the risk of severe COVID-19. *J Travel Med*. 2020. vol. 3.
5. Soler MJ, Barrios C, Oliva R, Batlle D. Pharmacologic modulation of ACE2 expression. *Curr Hypertens Rep*. Oct 2008;10(5):410-4. doi:10.1007/s11906-008-0076-0
6. Esler M, Esler D. Can angiotensin receptor-blocking drugs perhaps be harmful in the COVID-19 pandemic? *J Hypertens*. May 2020;38(5):781-782. doi:10.1097/hjh.0000000000002450
7. Sama IE, Ravera A, Santema BT, et al. Circulating plasma concentrations of angiotensin-converting enzyme 2 in men and women with heart failure and effects of renin-angiotensin-aldosterone inhibitors. *Eur Heart J*. May 14 2020;41(19):1810-1817. doi:10.1093/eurheartj/ehaa373
8. Reynolds HR, Adhikari S, Pulgarin C, et al. Renin-Angiotensin-Aldosterone System Inhibitors and Risk of Covid-19. *N Engl J Med*. Jun 18 2020;382(25):2441-2448. doi:10.1056/NEJMoa2008975
9. Mancia G, Rea F, Ludergnani M, Apolone G, Corrao G. Renin-Angiotensin-Aldosterone System Blockers and the Risk of Covid-19. *N Engl J Med*. Jun 18 2020;382(25):2431-2440. doi:10.1056/NEJMoa2006923
10. Gurwitz D. Angiotensin receptor blockers as tentative SARS-CoV-2 therapeutics. *Drug Dev Res*. Aug 2020;81(5):537-540. doi:10.1002/ddr.21656
11. Lopes RD, Macedo AVS, de Barros ESPGM, et al. Effect of Discontinuing vs Continuing Angiotensin-Converting Enzyme Inhibitors and Angiotensin II Receptor Blockers on Days Alive and Out of the Hospital in Patients Admitted With COVID-19: A Randomized Clinical Trial. *Jama*. Jan 19 2021;325(3):254-264. doi:10.1001/jama.2020.25864
12. Cohen JB, Hanff TC, William P, et al. Continuation versus discontinuation of renin-angiotensin system inhibitors in patients admitted to hospital with COVID-19: a prospective, randomised, open-label trial. *Lancet Respir Med*. Mar 2021;9(3):275-284. doi:10.1016/s2213-2600(20)30558-0
13. Cadegiani FA, Goren A, Wambier CG. Spironolactone may provide protection from SARS-CoV-2: Targeting androgens, angiotensin converting enzyme 2 (ACE2), and renin-angiotensin-aldosterone system (RAAS). *Med Hypotheses*. Oct 2020;143:110112. doi:10.1016/j.mehy.2020.110112
14. Pellicori P, Ferreira JP, Mariottoni B, et al. Effects of spironolactone on serum markers of fibrosis in people at high risk of developing heart failure: rationale, design and baseline characteristics of a proof-of-concept, randomised, precision-medicine, prevention trial. The Heart OMics in AGing (HOMAGE) trial. *Eur J Heart Fail*. Jan 16 2020;doi:10.1002/ejhf.1716
15. Cleland JGF, Ferreira JP, Mariottoni B, et al. The effect of spironolactone on cardiovascular function and markers of fibrosis in people at increased risk of developing heart failure: the heart 'OMics' in AGEing (HOMAGE) randomized clinical trial. *Eur Heart J*. Nov 20 2020;doi:10.1093/eurheartj/ehaa758
16. Wallentin L, Lindbäck J, Eriksson N, et al. Angiotensin-converting enzyme 2 (ACE2) levels in relation to risk factors for COVID-19 in two large cohorts of patients with atrial fibrillation. *Eur Heart J*. Nov 1 2020;41(41):4037-4046. doi:10.1093/eurheartj/ehaa697

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

	Plasma ACE2 concentration at baseline			
Characteristics	**Tertile 1**	**Tertile 2**	**Tertile 3**	**p-value**
N.	172	172	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
Women	71 (41%)	36 (21%)	25 (15%)	
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
ACEi	96 (56%)	81 (47%)	94 (55%)	0.21
ARB	44 (26%)	47 (27%)	50 (29%)	0.77
CCB	38 (22%)	37 (22%)	34 (20%)	0.86
Thiazides	31 (18%)	27 (16%)	28 (16%)	0.83
Statin	134 (78%)	137 (80%)	154 (90%)	0.010
Body Mass Index, Kg/m2	27 (24, 30)	28 (25, 31)	29 (27, 32)	<0.001
SBP, mmHg	140 (126, 157)	140 (126, 155)	140 (130, 153)	0.84
DBP, 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
LVEF, %	63 (59, 67)	62 (57, 66)	63 (58, 67)	0.42
LVM (BSA indexed), g/m2	92 (79, 110)	93 (81, 115)	97 (84, 112)	0.19
LAV (BSA indexed), ml/m2	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
TAPSE, mm,	23 (17, 27)	22 (18, 26)	22 (16, 26)	0.45
MAPSE, mm	16 (14, 18)	15 (13, 17)	15 (13, 18)	0.16
eGFR, ml/min/1.73m2	72 (60, 84)	73 (63, 83)	72 (61, 89)	0.75
Hemoglobin, 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 BNP, 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
PICP, ng/mL	78 (65, 95)	80 (64, 98)	81 (70, 100)	0.38
PIIINP, ng/ml	3.7 (3.0, 4.6)	3.9 (3.0, 5.0)	4.2 (3.3, 5.3)	0.004
CITP, ng/ml	3.7 (2.9, 4.9)	3.7 (2.8, 4.9)	3.8 (2.8, 5.0)	0.99

Legend: ACEi, angiotensin converting enzyme inhibitors; ARBs, angiotensin receptor blockers; CCB, calcium channel blockers; SBP, systolic blood pressure; DBP, diastolic blood pressure; LVEF, left ventricular ejection fraction; LVM, left ventricular mass; LAV, let atrial volume; eGFR, estimated glomerular filtration rate; PICP, procollagen type I carboxy-terminal propeptide; PIIINP, procollagen type III N-terminal propeptide; CITP, carboxy-terminal telopeptide of type I collagen; ACE2, angiotensin-converting enzyme 2.

Figure 1. Change in circulating ACE2 levels in patients taking spironolactone or usual care without spironolactone.



Caption: Spironolactone did not significantly change ACE2 levels. P-value =0.083 at month 1 and P-value =0.44 at month 9.