SHORT COMMUNICATION

Influence of renin-angiotensin-aldosterone system inhibitors on plasma levels of angiotensin-converting enzyme 2

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Abstract

Aims   Concern has been raised that treatment with angiotensin-converting enzyme inhibitors and angiotensin receptor blockers may increase the expression of angiotensin-converting enzyme 2 (ACE2), which acts as the entry receptor for SARS-CoV-2, and lead to an increased risk of death from SARS-CoV-2. We aimed to address this concern by evaluating the *in vivo* relationship of treatment with ACE inhibitors and angiotensin receptor blockers (ARB) with circulating plasma concentrations of ACE2 in a large cohort of patients with established cardiovascular disease (n = 1864) or cardiovascular risk factors (n = 2144) but without a history of heart failure.

Methods and results   Angiotensin-converting enzyme 2 was measured in 4008 patients (median age 68, 33% women, 31% on ACE-inhibitors, 31% on ARB) using the SOMAscan proteomic platform (SomaLogic Inc, Colorado, USA). Plasma concentration of ACE2 was comparable in 1250 patients on ACE inhibitors (mean 5.99) versus patients without ACE inhibitors (mean 5.98, P = 0.54). Similarly, plasma concentration of ACE2 was comparable in 1260 patients on ARB (mean 5.99) versus patients without ARB (mean 5.98, P = 0.50). Plasma concentration of ACE2 was comparable in 2474 patients on either ACE inhibitors or ARB (mean 5.99) versus patients without ACE inhibitors or ARB (mean 5.98, P = 0.31). Multivariable quantile regression model analysis confirmed the lack of association between treatment with ACE inhibitors or ARB and ACE2 concentrations. Body mass index showed the only positive association with ACE2 plasma concentration (effect 0.015, 95% confidence interval 0.002 to 0.028, P = 0.024).

Conclusions   In a large cohort of patients with established cardiovascular disease or cardiovascular risk factors but without heart failure, ACE inhibitors and ARB were not associated with higher plasma concentrations of ACE2.

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Background

The majority of deaths from the new severe acute respiratory syndrome coronavirus (SARS-CoV-2) occurred in patients with cardiovascular disease, who often were on chronic treatment with angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARB).[1] This epidemiological association together with experimental evidence that treatment with ACE inhibitors and ARB may increase the expression of angiotensin-converting enzyme 2 (ACE2), which acts as the entry receptor for SARS-CoV-2, has led to concern that ACE inhibitors and ARB may causally contribute to an increased risk of death from SARS-CoV-2 via increasing ACE2.[2]

Aims

We aimed to address this concern by evaluating the *in vivo* effect of treatment with ACE inhibitors and ARB on circulating plasma concentrations of ACE2 in a large cohort of patients with established cardiovascular disease (*n* = 1864) or cardiovascular risk factors (*n* = 2144), but without a history of heart failure (NCT01838148, ClinicalTrials.gov).[3]

Methods

This analysis was performed in a large prospective diagnostic study (ClinicalTrials.gov, NCT01838148) designed to advance the early detection of inducible myocardial ischaemia. Consecutive adult patients with symptoms possibly related to inducible myocardial ischaemia, who were referred for stress and rest myocardial perfusion imaging with single-photon emission computed tomography combined with computed tomography (MPI-SPECT/CT) to the University Hospital Basel (Basel, Switzerland), were recruited. All patients provided written informed consent. Clinical information, including patient characteristics, medication, symptoms, and cardiovascular history, was documented by physicians using standardized questionnaires and all medical files available. The study was approved by the local ethics committee and carried out according to the principles of the Declaration of Helsinki.

Of the 4219 patients enrolled in the study, ACE2 was measured in 4008 patients (median age 68 [interquartile range 60 to 76], 33% women, 31% on ACE inhibitors, 31% on ARB, baseline characteristics; see *Table 1*) using the SOMAscan proteomic platform version 4 (SomaLogic Inc, Colorado, USA).[4] In brief, SOMAscan uses a proprietary DNA-based aptamer technology to bind to the target protein with high

Table 1 Baseline characteristics of the overall study cohort and stratified by the use of ACEi or ARB

Variable	Overall	No ACEi or ARB	ACEi or ARB	P
n	4008	1534	2474	
Age (years)	68.0 [60.0, 76.0]	66.0 [57.0, 74.0]	69.0 [61.0, 76.0]	<0.001
Female	1316 (33)	584 (38)	732 (30)	<0.001
eGFR (mL/min/1.73 m^2)	81.0 [61.4, 93.0]	85.6 [69.7, 96.5]	77.6 [57.1, 91.0]	<0.001
BMI (kg/m^2)	27.1 [24.4, 30.5]	26.1 [23.7, 29.4]	27.7 [24.9, 31.2]	<0.001
Medical history				
Atrial fibrillation	561 (14)	172 (11)	389 (16)	<0.001
Diabetes	995 (25)	240 (16)	755 (31)	<0.001
Arterial hypertension	3227 (81)	762 (50)	2465 (100)	<0.001
Chronic obstructive pulmonary disease	324 (8)	108 (7)	216 (9)	0.057
Myocardial infarction	1156 (29)	237 (15)	919 (37)	<0.001
Coronary artery disease	1864 (47)	480 (31)	1384 (56)	<0.001
Percutaneous coronary intervention	1420 (35)	358 (23)	1062 (43)	<0.001
Coronary artery bypass graft	532 (13)	140 (9)	392 (16)	<0.001
Medication				
ACE inhibitors	1250 (31)	-	1250 (51)	<0.001
Angiotensin receptor blockers	1260 (31)	-	1260 (51)	<0.001
Beta-blockers	2226 (56)	659 (43)	1567 (63)	<0.001
Diuretics	1546 (39)	230 (15)	1316 (53)	<0.001
Calcium channel blockers	926 (23)	215 (14)	711 (29)	<0.001

ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; eGFR, estimated glomerular filtration rate.

specificity and transforms individual protein concentrations into a corresponding modified aptamer concentration. Therefore, resulting concentrations [relative fluorescent units (RFU)] are relative and directly proportional to the amount of target protein in the initial sample. Additional analytical validation was performed in this study by utilizing routine cardiovascular biomarker measurements [n-terminal pro-B-type natriuretic peptide (NT-proBNP), Spearman ρ = 0.63 and growth differentiation factor 15 (GDF-15), Spearman ρ = 0.93, both $P < 0.001$, both Roche Diagnostics] for correlation analysis with SOMAscan results. The natural logarithm of ACE2 measurements was used for all analyses.
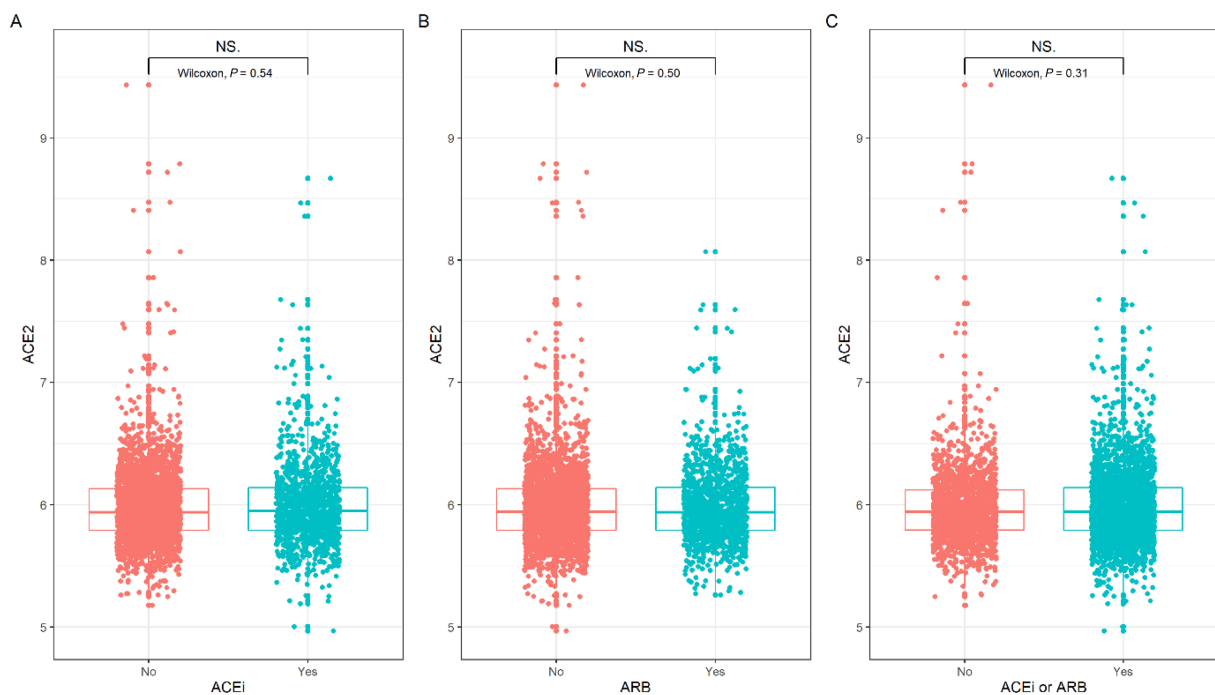
diabetes, arterial hypertension, chronic obstructive pulmonary disease, myocardial infarction, coronary artery disease, percutaneous coronary intervention, and coronary artery bypass graft confirmed the lack of association between treatment with ACE inhibitors or ARB and ACE2 concentrations.

Body mass index was positively associated with ACE2 plasma concentration (effect 0.015, 95% CI: 0.002 to 0.028, P = 0.024), while age (-0.049, 95% CI: -0.065 to -0.034, $P < 0.001$), history of atrial fibrillation (-0.034, 95% CI: -0.062 to -0.006, P = 0.024), history of PCI (-0.041, 95% CI: -0.080 to -0.001, P = 0.042), and history of CABG (-0.052, 95% CI: -0.081 to -0.022, $P < 0.001$) were all negatively associated with ACE2 concentrations in the model.

Results

Plasma concentration of ACE2 was comparable in 1250 patients on ACE inhibitors (mean 5.99) versus patients without ACE inhibitors (mean 5.98, P = 0.54; *Figure 1*). Similarly, plasma concentration of ACE2 was comparable in 1260 patients on ARB (mean 5.99) versus patients without ARB (mean 5.98, P = 0.50). Also, plasma concentration of ACE2 was comparable in 2474 patients on either ACE inhibitors or ARB (mean 5.99) versus patients without ACE inhibitors or ARB (mean 5.98, P = 0.31).

Multivariable quantile regression model analysis adjusted for age, sex, body mass index, history of atrial fibrillation,

Discussion/conclusion

This large observational study found no association between treatment with ACE inhibitors and/or ARB and circulating plasma concentrations of ACE2 in patients with established cardiovascular disease or cardiovascular risk factors, but without overt heart failure, representing the population most severely affected by SARS-CoV-2. This finding extends and corroborates similar observations regarding the lack of associations between treatment with ACE inhibitors and/or ARB on ACE2 plasma concentrations in two independent cohorts of patients with overt heart failure.[5,6] The first study included

Figure 1 ACE2 concentrations in patients stratified by use of ACEi and/or ARB. ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; ACE2, angiotensin-converting enzyme 2; NS, non-significant.

1485 men and 537 women with heart failure in the derivation cohort and 1123 men and 575 women in the validation cohort, all enrolled in Europe. Median age was 69 years for men and 75 years for women. The second study included 2248 patients with heart failure enrolled in the USA.[5,6] In contrast to the European heart failure study,[5] this study did not find male sex to be a significant predictor for ACE2 plasma levels. This might in part be explained by an upregulation of ACE2 expression in heart-failure patients,[7] potentially more so in men than in women.[8] Overall, these biomarker studies are in agreement with observational clinical studies documenting a lack of increased risk of severe illness in patients with COVID-19 treated with an ACE inhibitor or ARB.[9] However, ACE2 regulation by RAAS blockade might be different in patients with active COVID-19 compared with patients without, and further research is needed on this topic.

Taken together with emerging epidemiological data,[1] the three *in vivo* studies on ACE2 plasma concentrations support the concept that treatment with ACE inhibitors or ARB should not be withheld from patients at risk for infection with SARS-CoV-2, although this will need to be ultimately clarified by clinical trials.[10,11]

Some limitations should be considered. First, liquid chromatography coupled to mass spectrometry is the gold standard for plasma ACE2 analysis. Therefore, it is reassuring that SOMAscan measurements were shown to be largely in accordance with proteome and transcriptome measurements and the ACE2 aptamer specifically has been validated.[6,12] Second, like previous studies, we measured circulating ACE2 plasma concentrations. The equilibrium between circulating and membrane-bound ACE2 remains, to our knowledge, incompletely understood.

In conclusion, in a large cohort of patients with established cardiovascular disease or cardiovascular risk factors but without heart failure, ACE inhibitors and ARB were not associated with higher plasma concentrations of ACE2.

Conflict of interest

Funding

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