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Roles of Angiotensin Peptides and Recombinant Human ACE2 in Heart Failure

Ratnadeep Basu, MD, PhD,[a,b] Marko Poglitsch, PhD,[c] Haran Yogasundaram, MD,[a,b] Jissy Thomas, MN,[b] Brian H. Rowe, MD,[d] Gavin Y. Oudit, MD, PhD[a,b]

ABSTRACT

BACKGROUND The renin-angiotensin system (RAS) is activated in heart failure (HF) and inhibition of RAS is a mainstay therapy for HF. Angiotensin-converting enzyme 2 (ACE2) and its product, angiotensin 1–7 (Ang-[1–7]), are important negative regulators of the RAS.

OBJECTIVES A comprehensive examination of angiotensin peptide levels and therapeutic effects of recombinant human ACE2 (rhACE2) on peptide metabolism was evaluated in human plasma and explanted heart tissue from patients with HF.

METHODS Using prospective cohorts with chronic (n = 59) and acute (n = 42) HF, plasma angiotensin analysis was performed using a unique liquid chromatography-mass spectrometry/mass spectroscopy method quantifying circulating and equilibrium levels. Angiotensin II (Ang II) metabolism was examined in human explanted hearts with dilated cardiomyopathy (n = 25).

RESULTS The dynamic range of the RAS was large, with equilibrium angiotensin levels being 8- to 10-fold higher compared with circulating angiotensin levels. In chronic HF patients receiving ACE inhibition, plasma Ang II was suppressed and plasma Ang-(1–7) was elevated, whereas acute HF and patients receiving angiotensin receptor blocker had higher plasma Ang II with lower Ang-(1–7) levels. Suppressed Ang-(1–7)/Ang II ratio was associated with worsening HF symptoms and longer hospitalization. Recombinant human ACE2 effectively metabolized Ang-(1–10) and Ang II into Ang-(1–9) and Ang-(1–7), respectively. Myocardial Ang II levels in explanted human hearts with dilated cardiomyopathy were elevated despite ACE inhibition with elevated chymase activity, and Ang II was effectively converted to Ang-(1–7) by rhACE2.

CONCLUSIONS Plasma angiotensin peptides represent a dynamic network that is altered in HF and in response to rhACE2. An increased plasma Ang-(1–7) level is linked to ACE inhibitor use, whereas acute HF reduced Ang-(1–7) levels and suppressed the Ang-(1–7)/Ang II ratio. Increased chymase activity elevated Ang II levels in failing human hearts. Use of rhACE2 effectively normalized elevated Ang II while increasing Ang-(1–7) and Ang-(1–9) levels.
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Despite improvement in survival of patients with heart failure (HF) (1), mortality and morbidity remain high, resulting in an enormous health and economic burden (2). Moreover, a tailored biomarker approach enabling precision medicine might be possible if our understanding of HF pathophysiology can be improved (3). Activation of the renin-angiotensin system (RAS) leading to elevated plasma and tissue angiotensin II (Ang II) levels is a well-established neurohumoral basis

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From the [a]Division of Cardiology, Department of Medicine, University of Alberta, Edmonton, Canada; [b]Mazankowski Alberta Heart Institute, University of Alberta, Edmonton, Canada; [c]Attoquant Diagnostics, Vienna, Austria; and the [d]Department of Emergency Medicine and School of Public Health, University of Alberta, Edmonton, Canada. Funding support was provided by the Canadian Institutes of Health Research (CIHR), Alberta Innovates-Health Solutions (AI-HS), Heart and Stroke Foundation (HSF), and the University of Alberta Hospital Foundation. Dr. Rowe's research is supported by a Tier I Canada Research Chair in Evidence-Based Emergency Medicine and Dr. Oudit's research is supported by a Tier II Canada Research Chair in Heart Failure through the Government of Canada (Ottawa, Ontario). Dr. Poglitsch is an employee of Attoquant Diagnostics. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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ABBREVIATIONS AND ACRONYMS

ACE = angiotensin-converting enzyme

ACEI = angiotensin-converting enzyme inhibitor

AHF = acute heart failure

Ang I = angiotensin-(1-10)

Ang II = angiotensin-(1-8)

ARB = angiotensin receptor blocker

CHF = chronic heart failure

EF = ejection fraction

HC = healthy control

HF = heart failure

HFpEF = heart failure with preserved ejection fraction

IQR = interquartile range

LLOQ = lower limit of quantification

NFC = nonfailing control

NYHA = New York Heart Association

RAS = renin-angiotensin system

rhACE2 = recombinant human ACE2

underlying the pathogenesis of HF (4,5). Targeting the RAS axis, primarily in the form of angiotensin-converting enzyme (ACE) inhibitors (ACEI) (6–8) and angiotensin receptor blockers (ARB) (8–10) reduces mortality and morbidity in patients with HF. However, irrespective of the capacity of ACEIs to inhibit ACE action, Ang II levels can remain elevated in optimally treated HF patients (11,12). Increased plasma Ang II, despite ACEI therapy, is a significant predictor of death and HF (13). Heterogeneity in the HF population warrants exploration of potential important roles of other peptide systems and the potential for tailored drug treatments.

Angiotensin-converting enzyme 2 (ACE2), a negative regulator of the RAS axis, catalyzes the vasoconstrictive, proinflammatory, and fibrogenic Ang II peptide to the vasodilatory, anti-inflammatory, and antifibrogenic angiotensin-(1-7) (Ang-[1-7]) peptide (14–17). Increasing Ang-(1-7) levels improves heart function and reverses pathological remodeling in preclinical models of HF (15–18). Elevated plasma ACE2 activity in HF patients (19), suppression of cardiac hypertrophy and dysfunction in preclinical models by recombinant human ACE2 (rhACE2) (16,17), and the successful use of rhACE2 in healthy volunteers (20) have highlighted the potential therapeutic potential of modulating the ACE2/Ang-(1-7) axis in HF. The intricate balance between various peptides and peptidases, which constitute the RAS axis, is incompletely understood in the context of contemporary chronic stable and acute decompensated HF patients. Our study extensively profiled plasma RAS peptides in patients with chronic and acute HF and tissue Ang II levels in explanted human hearts with dilated cardiomyopathy (DCM). Importantly, we examined the therapeutic potential of rhACE2 in both plasma and hearts.

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METHODS

This study was conducted in accordance with the ethical principles of the declaration of Helsinki convention and the guidelines as approved by the Health Research Ethics Board of the University of Alberta. Written informed consent was obtained from all subjects.

A single-center prospective screening study was performed at the Mazankowski Alberta Heart Institute and the emergency department at the University of Alberta Hospital. Stable ambulatory patients with New York Heart Association (NYHA) functional class I/II HF, who were optimally treated with either ACEI (n = 42) or ARB (n = 17) therapy were recruited from the heart function clinic; plus, patients with acute decompensated heart failure (AHF) (functional class III/IV; n = 42) who were admitted after emergency department presentation, were enrolled as were age- and sex-equivalent healthy controls (n = 36). Any patients with investigational drug use within the last 4 weeks were excluded.

Venous blood sampling was performed by trained phlebotomists at approximately the same time of day (10:00 am to 1:00 pm) to avoid any diurnal variation of measured peptide levels. Additional details about blood collection are in the Online Appendix.

PEPTIDE AND ENZYME MEASUREMENT AND ANALYSIS. Circulating Ang peptide concentrations were determined by mass spectrometry using plasma samples collected in the presence of an inhibitor cocktail completely blocking angiotensin metabolism (Online Appendix). Equilibrium angiotensin peptide levels were measured following 30 min of equilibration of conditioned lithium-heparin plasma at 37°C and subsequent stabilization of equilibrium peptide levels as described previously (20). Stabilized protease inhibitor and equilibrated samples were further spiked with stable isotope-labeled internal standards for each angiotensin metabolite, angiotensin-(1-10) (Ang I), Ang II, Ang-(1-7), Ang-(1-5), Ang-(2-8), Ang-(3-8), Ang-(2-10), Ang-(2-7), Ang-(1-9), and Ang-(3-7) at a concentration of 200 pg/ml. Following C18-based solid-phase-extraction, samples were subjected to liquid chromatography-mass spectrometry/mass spectroscopy (LC-MS/MS) analysis using a reversed-phase analytical column operating in line with a Xevo TQ-S triple quadrupole mass spectrometer (Waters, Milford, Massachusetts). Internal standards were used to correct for peptide recovery of the sample preparation procedure for each angiotensin metabolite in each individual sample. Ang peptide concentrations were calculated considering the corresponding response factors determined in appropriate calibration curves in original sample matrix, on condition that integrated signals exceeded a signal-to-noise ratio of 10. The lower limit of quantification of the circulating and equilibrium levels of the individual angiotensin peptides and the recovery rates of the various peptides are reported in Online Table 1.

Aldosterone levels were measured quantitatively in human plasma collected in lithium heparin-containing vials, using a chemiluminescence immunoassay (Immunodiagnostic Systems, Gaithersburg,