Clinical Investigations

Plasma Corin Levels Provide Minimal Prognostic Utility Incremental to Natriuretic Peptides in Chronic Systolic Heart Failure

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ABSTRACT

Background: Corin is a serine protease that cleaves pro-atrial and pro-B-type natriuretic peptides into biologically active hormones. The relationship between soluble plasma corin levels, plasma natriuretic peptide levels, myocardial structure and performance, and long-term clinical outcomes in the setting of chronic systolic heart failure has not been described.

Methods and Results: In 126 patients with chronic systolic heart failure (left ventricular ejection fraction ≤35%, New York Heart Association functional Class I-IV), we measured plasma corin and natriuretic peptide levels and performed comprehensive echocardiography with assessment of cardiac structure and performance. Adverse clinical events (all-cause mortality, cardiac transplantation, or heart failure hospitalization) were prospectively tracked for a median of 38 months. Plasma corin levels modestly correlated with echocardiographic indices of cardiac structure, including left ventricular mass index ($r = 0.30$, $P = .003$) and interventricular septum width ($r = 0.22$, $P = .013$). However, plasma corin levels did not correlate with age, arterial pressures, estimated glomerular filtration rate, echocardiographic indices of systolic or diastolic function, or plasma natriuretic peptide levels. In Cox proportional hazards analysis, higher plasma corin levels did not predict reduced risk of adverse clinical events (hazard ratio 0.91; 95% confidence interval 0.67-1.24, $P = .52$), and did not provide incremental prognostic value to natriuretic peptide levels.

Conclusion: In our cohort of ambulatory patients with chronic systolic heart failure, soluble plasma corin levels did not provide prognostic utility incremental to that of natriuretic peptides. (*J Cardiac Fail 2010;16:621−627*)

Key Words: Congestive heart failure, corin, natriuretic peptides, ventricular hypertrophy.

Natriuretic peptides play an important role in heart failure, antagonizing the renin-angiotensin system as well as promoting natriuresis, diuresis, and vasodilation in response to volume and pressure overload. In addition to hemodynamic load, natriuretic peptide expression and secretion are upregulated in response to diverse cardiac pathophysiologic stressors including ischemia, ventricular hypertrophy, and chronic neurohumoral activation.[1] Furthermore, natriuretic peptides have been shown to suppress cardiac fibrosis and hypertrophy through pressure-independent mechanisms.[1-4] Both atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) are initially synthesized in atrial and ventricular cardiomyocytes as ''pre-prohormones.'' Removal of their amino-terminal signal sequences yields the prohormones, pro-ANP and pro-BNP, which have been reported to have minimal biological activity but serve as immediate substrates for the production of the active hormones.[1]

Corin, a type II transmembrane serine protease highly expressed in cardiomyocytes, has recently been established

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as the physiologic pro-ANP convertase, cleaving pro-ANP into aminoterminal (NT)-proANP and biologically active ANP.[5-7] In cell culture studies, corin has also been shown to cleave pro-BNP into NT-proBNP and active BNP.[6] In patients with heart failure, markedly increased levels of inactive pro-ANP and pro-BNP relative to active ANP and BNP levels have been reported, leading some to characterize heart failure as a natriuretic peptide ''processing-deficient'' state.[8] Recent cell culture and animal experimental models have further suggested that expression of corin may be altered in the setting of heart failure.[9,10] Although there are increasing data to implicate the role of corin expression in the pathogenesis of hypertension, the overall clinical importance of corin in the pathophysiology of human heart failure remains incompletely understood.

Recently, detectable circulating corin levels have been reported.[11] Considering many hormonal systems can shed their transmembrane enzymes as a compensatory mechanism in disease states, the extent to which corin circulates in plasma to alter natriuretic peptide processing beyond the cell membrane has not been described. The goal of this study is to examine the role of corin levels detected in plasma samples of ambulatory patients with chronic systolic heart failure in relation to clinical and echocardiographic parameters, other circulating natriuretic peptide levels, as well as long-term adverse cardiac events.

Methods

Study Design and Population

This analysis is part of the neurohumoral substudy of the Assessment of Doppler Echocardiography in Prognosis and Therapy study, a single-center, prospective cohort study that was approved by the Cleveland Clinic Institutional Review Board. Briefly, 126 ambulatory subjects with stable but symptomatic, chronic systolic heart failure (left ventricular [LV] ejection fraction ≤35%, New York Heart Association functional Class I-IV) underwent echocardiographic evaluation of systolic and diastolic performance with plasma sample collection. These 126 subjects were included from a subset of the 160 consecutive subjects enrolled in the original Assessment of Doppler Echocardiography in Prognosis and Therapy neurohumoral substudy according to sample availability. Estimated glomerular filtration rate was calculated using the standard 4-variable Modification of Diet in Renal Disease equation.[12] The composite endpoint of adverse clinical events (including all-cause mortality, cardiac transplantation, or heart failure hospitalization) was prospectively tracked for a median of 38 months by telephone follow-up and medical chart review as previously described.[13]

Transthoracic Echocardiography

Comprehensive transthoracic echocardiography was performed using commercially available HDI 5000 (Phillips Medical Systems, N.A., Bothell, WA) and Acuson Sequoia (Siemens Medical Solutions USA Inc, Malvern, PA) machines. Two-dimensional and color Doppler imaging was performed in standard parasternal and apical views. Diastolic indices (including pulse-wave Doppler, color M-mode, and tissue Doppler imaging) were acquired over 10 consecutive beats using sweep speeds of 50 and 100 cm/s using previously described techniques.[13,14] Classification of diastolic stage was determined as follows: (1) *Stage I (impaired relaxation)* consists of mitral E/A <1, deceleration time (DT) >220 ms, pulmonary vein S/D >1, color M-mode propagation velocity <45 cm/s; (2) *Stage II (pseudonormal)* shows mitral E/A 1-2, pulmonary vein S/D <1, DT <220 ms, propagation velocity <45 cm/s; (3) *Stage III (restrictive)* gives mitral E/A >2, pulmonary vein S/D <1, DT <150 ms, propagation velocity <45 cm/s. The LV ejection fraction and cardiac volumes were measured using Simpson's biplane method. LV mass was calculated according to previously published recommendations.[15] All ventricular volume and mass measurements were indexed to body surface area. Measurements were averaged over 3 cycles (5 cycles for atrial fibrillation), and measured by 2 experienced individuals before analyses of the neurohormonal data.

Plasma Corin and Natriuretic Peptide Measurement

All samples were collected into ethylenediaminetetraacetic acid-plasma vacuum collecting tubes on ice simultaneously at the time of echocardiography by delegated research personnel. Plasma was immediately separated, processed, and preserved in aliquots at −80°C.

Plasma corin levels were determined by a commercial sandwich enzyme-linked immunosorbent assay (Cat. No. DCRN00, Quantikine, R&D Systems, Minneapolis, MN) using a 2-fold sample dilution. In linearity testing, percentage of expected recovery over the dynamic range of the assay (1:2 to 1:16 dilution) fell within the range of 86% to 105%. At a 1:2 dilution, the average percentage of expected recovery was 100% with a range of 99% to 102%. No significant cross-reactivity or interference was observed (<1%). This assay demonstrated a minimum detection limit of 1.6 pg/mL. All diluted samples fell within the range of the corin calibration standard curve (75 to 4800 pg/mL). Intra-assay and inter-assay coefficients of variation were <5% at 600 pg/mL.

Plasma BNP, NT-proANP, and ANP levels were assayed using a validated radioimmunoassay developed by the Christchurch Cardioendocrine Research Group that has been previously described.[16,17] The natriuretic peptides BNP, ANP, and NT-proANP were extracted on sep-pak C18 cartridges equilibrated with 0.1% trifluoroacetic acid. Plasma was loaded and the cartridges washed with of 0.1% trifluoroacetic acid and eluted with 2 mL of 80% isopropanol/0.1% trifluoroacetic acid. The eluate was dried and reconstituted in assay buffer. The ANP assay was performed as previously described,[16] except that a more sensitive locally raised antiserum (R31) was used providing a detection limit of 9 pmol/L (2 pmol/L in plasma after extraction). Intra-assay and inter-assay coefficients of variation at 28 pmol/L were both 8%. Cross-reactivities were human ANP 100%, human BNP (hBNP) 0.05%, and CNP-22 <0.13%. Although not measured, we expect this assay to cross-react with proANP. The NT-proANP assay was performed as previously described for ANP,[16] except that that an antiserum to proANP(1-30), a proANP(1-30) standard (Bachem, Bubendorf, Switzerland) and radiolabeled proANP(1-30) were used. These reagents were added together and incubated at 4 °C for 24 hours before separation of bound and free label. The assay had a detection limit in plasma of 10 pmol/L and intra-assay and inter-assay coefficients of variation at 1370 pmol/L were 12% and 14%, respectively. hBNP, human ANP, hCNP-22, and proBNP(1-3) had undetectable cross-reactivity in the assay. The BNP radioimmunoassay was performed as previously described[17] and employed an antiserum to hBNP (Bachem). This antiserum had the following