Genetic and Nongenetic Factors Influencing Pharmacokinetics of B-Type Natriuretic Peptide

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ABSTRACT

Background: Natriuretic peptides (NPs) represent a critical pathway in heart failure (HF). However, there is wide individual variability in NP system activity, which could be partly genetic in origin. We explored genetic and nongenetic contributions to B-type natriuretic peptide (BNP) inactivation.

Methods: Chronic HF patients (n = 95) received recombinant human BNP (nesiritide) at standard doses, and BNP levels were measured at baseline, after 2 hours of infusion, and 30 minutes after discontinuation. Genomic DNA was genotyped for 91 single-nucleotide polymorphisms (SNP) in 2 candidate genes. We tested the association of patient characteristics and genotype with 5 pharmacokinetics (PK) parameters: elimination rate constant, ΔBNP, BNP clearance, adjusted BNP clearance, and half-life. Linear regression with pleiotropic analysis was used to test genotype associations with PK.

Results: Participants' mean age was 63 years, 44% were female, and 46% were African American. PK parameters varied widely, some >10-fold. HF type (preserved vs reduced) was associated with PK ($P < .01$), whereas renal function, demographics, and body mass index and were not. Two SNPs in *MME* (rs989692, rs6798179) and 2 in *NPR3* (rs6880564, rs2062708) also had associations with PK ($P < .05$).

Conclusions: The pharmacokinetics of BNP varies greatly in HF patients, differs by HF type, and possibly by *MME* or *NPR3* genotype. Additional study is warranted. (*J Cardiac Fail 2014;20:662−668*)

Key Words: Natriuretic peptide, heart failure, drug metabolism, pharmacogenetics, genetic polymorphisms.

Heart failure (HF) continues to be an enormous public health problem despite the many advances in pharmacotherapy over the past 25 years, with a prevalence of 5.7 million individuals affected and an incidence of >500,000 new cases annually.[1] Therefore, better prognostics, improved targeting of current therapies, and novel therapies are still needed. The relevance of the natriuretic peptide (NP) system, particularly B-type NP (BNP), is well known in terms of HF pathophysiology,[2,3] diagnosis,[4] prognosis,[5] and therapy.[6,7] Moreover, the response to extrinsic NP (eg, nesiritide, carperitide) is also highly variable, with unclear therapeutic range and potential for adverse effects.[8−10] A portion of this variation in NP system functioning is likely to be genetic in origin,[11] as evidenced by clear associations of genetic variants with cardiovascular disease states,[12,13] altered gene expression and protein abundance,[14,15] as well as native NP levels via altered production or elimination.[16−18] NP system variability will continue to be a relevant clinical issue not only because our understanding of NP system biology is still incomplete, but also because BNP is being increasingly explored in personalized care[19] and because there are numerous investigational NP therapeutics currently under development.[20,21]

We sought to systematically assess the genetic and nongenetic associations of BNP clearance by infusing intravenous recombinant human BNP (nesiritide), quantifying key

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pharmacokinetics (PK) parameters, and focusing on common sequence variation in candidate genes relevant to BNP metabolism. The 2 primary molecular clearance mechanisms previously identified for NPs are membrane metalloendopeptidase (also known as neutral endopeptidase), encoded by the gene *MME*, and natriuretic peptide receptor C, encoded by the *NPR3* gene (Fig. 1). MME enzymatically degrades natriuretic (and other) peptides to predominantly inactive forms. NPRC is a noncatalytic NP receptor which shares homology with the other NP receptors in the transmembrane portion but lacks the intracellular guanylate cyclase domain. It is thought that its primary role is in peptide internalization and clearance, although effector roles have recently been proposed.

We prospectively enrolled patients with HF who were planned to receive nesiritide infusion to evaluate the overall variation in BNP kinetics in a typical HF population, examine the association with clinical and demographic characteristics, and test the association of genetic variation in the 2 candidate genes with PK parameters. We hypothesized that genetic variation in key genes may be associated with differences in drug elimination. If true this could help to improve patient selection and dosing of NP-based therapeutics, as well as possibly affect the interpretation of native BNP levels and prognostication.

Methods

Patients and Sample Collection

The study was approved by the Henry Ford Hospital Institutional Review Board, and every patient provided written informed consent. Patients with a history of HF who were receiving nesiritide either as part of clinical care (hospitalized HF patients) or only for the purposes of the study (ambulatory chronic HF patients) were included. Subjects with baseline systolic blood pressure <110 mm Hg or with end-stage renal disease were excluded. Patients received intravenous nesiritide at standard doses: 2 μg/kg bolus followed by 0.01 μg kg^{-1} min^{-1} continuous infusion. Patients were monitored for 1 hour before the bolus and were monitored for an additional 2 hours after. Blood samples were drawn at baseline and after 2 hours of infusion. Blood pressure was monitored every 15 minutes throughout. For patients not receiving nesiritide as part of standard care during an episode of hospitalized HF, nesiritide was discontinued after the 2 hour blood drawing, and another level was checked after 30 minutes to assess drug half-life. All investigational patient samples were centrifuged, aliquotted, and frozen within 30 minutes. These were stored at $-70°C$ until batch testing could be performed at the Henry Ford Hospital clinical chemistry lab.

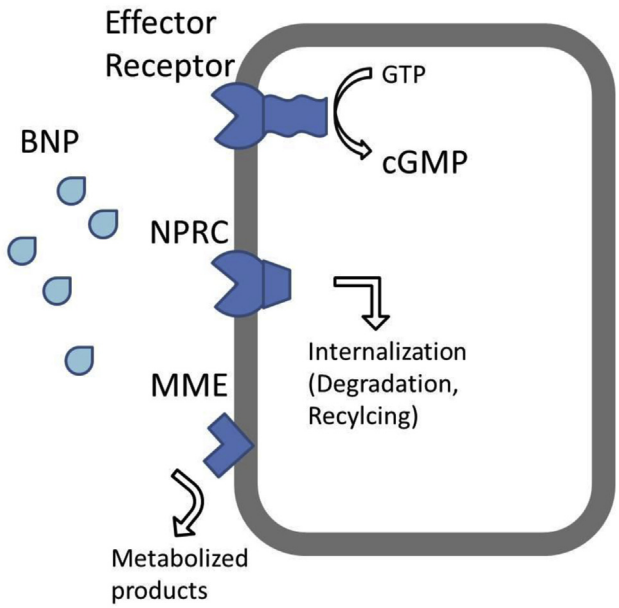

Fig. 1. Schematic diagram of B-type natriuretic peptide (BNP) processing. cGMP, cyclic guanosine monophosphate; GTP, guanosine triphosphate; MME, membrane metalloendopeptidase; NPRC, natriuretic peptide receptor C.

Genotyping

DNA samples were genotyped using a custom Illumina Goldengate array which contained candidate-gene coverage relevant to HF, including focused attention on the genes of interest to the NP pathway. Single-nucleotide polymorphisms (SNPs) were chosen for the array by attempting to include all coding variants, as well as with the use of HAPMAP to select optimal noncoding (''tag'') variants to capture blocks with minor allele frequency of >0.1 prevalence in whites or African Americans within the gene regions of interest. After processing requirements for the Goldengate technology and quality control of genotyping, 91 SNPs in the 2 candidate genes of interest (*MME,* 41 SNPs; *NPR3,* 50 SNPs) were available for analysis. Genotyping calls were made with the use of Genomestudio software automatic algorithms (Illumina, San Diego, California), and then individual SNPs were reviewed manually. Sample call rates were $>90\%$, and none of the SNPs analyzed deviated significantly from Hardy-Weinberg equilibrium.

B-Type Natriuretic Peptide Levels and Pharmacokinetics Parameters

B-Type natriuretic peptide levels were tested in plasma samples by Henry Ford Hospital clinical chemistry lab and personnel. The lab uses Centaur (Advia, Bayer Diagnostics), which has high precision (coefficient of variation $<5\%$) and is capable of directly measuring levels up to 5,000 ng/L (higher levels measured with dilution).[22] The upper limit reported by our laboratory is 8,000 ng/L after dilutions, which was assigned as the maximum possible BNP value. BNP levels were measured at 2 time points in all patients: baseline and after 2 hours of infusion. Two hours of infusion was chosen because it was thought to be representative of steady-state BNP level because this is >6 times the previously reported average half-life of ~ 18 minutes.[23] A third time point of 30 minutes after discontinuation of drug was also tested in patients where the nesiritide could be stopped (ie, those subjects who were not prescribed nesiritide for clinical purposes in the hospital). We then derived and tested 4 PK end points, calculated as follows:

1. Change in BNP level ($\Delta BNP = BNP_{SS} - BNP_B$)
2. Elimination rate constant ($K = [1{,}000{,}000 \times \text{infusion rate}]/[73 \times \Delta BNP]$)
3. BNP clearance rate ($CL = 1000 \times \text{infusion rate} \times \text{weight}/BNP_{SS}$)
4. BNP adjusted clearance rate ($CL = 1000 \times \text{infusion rate} \times \text{weight}/\Delta BNP$)
5. Half-life ($HL = -30[Ln(0.5)]/Ln(BNP_{SS}/BNP_D)$).