Natriuretic and Neurohormonal Responses to Nesiritide, Furosemide, and Combined Nesiritide and Furosemide in Patients With Stable Systolic Dysfunction

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ABSTRACT

Background: In patients with heart failure, few data describe the neurohormonal response to nesiritide and furosemide either alone or in combination. This study systematically compared the effects of nesiritide, furosemide, and their combination on natriuresis/diuresis and plasma aldosterone in patients with chronic stable heart failure who were relatively diuretic resistant.

Hypothesis: Natriuretic, diuretic, and neurohormonal responses to furosemide and nesiritide will differ when these agents are administered alone vs. in combination.

Methods: Twenty-eight subjects completed a multicenter, open-label, three-arm crossover study. Each subject received the following treatments in random order on alternate days: (1) furosemide, 40 mg intravenous bolus; (2) nesiritide, 2 µg/kg intravenous bolus followed by a 0.01 µg/kg/min infusion for 6 hours; (3) both furosemide and nesiritide, with furosemide given at least 15 minutes after initiation of nesiritide.

Results: Plasma aldosterone increased by 2.2 ± 1.6 ng/dL after furosemide alone, decreased by 3.9 ± 1.6 ng/dL after nesiritide alone ($P = 0.005$ vs furosemide alone and $P = 0.56$ vs furosemide plus nesiritide), and decreased by 2.8 ± 1.6 ng/dL after furosemide plus nesiritide ($P = 0.02$ vs furosemide alone).

Conclusions: Furosemide alone produced natriuresis/diuresis and a prompt rise in plasma aldosterone values. Nesiritide alone produced no significant natriuresis/diuresis, but decreased plasma aldosterone values. When furosemide was administered on a background of nesiritide infusion, the observed natriuresis/diuresis was similar to that seen with furosemide alone, without the anticipated increase in plasma aldosterone observed with furosemide alone.

Introduction

Physicians who manage patients with acute decompensated heart failure (ADHF) face many treatment problems, including the syndrome of “diuretic resistance.”¹ Diuretic resistance refers to attenuation of the expected dose-dependent increase in natriuretic response to a diuretic agent.² Current approaches to reducing excess total body

salt and water with diuretic agents in the setting of ADHF include measures intended to optimize loop diuretic delivery to the active site in the tubular compartment, while also attempting to enhance the pharmacodynamic action of that diuretic so delivered.^{1,2} Although few data support the practice, a number of compounds have been used clinically in attempts to improve loop diuretic-related diuresis, including combination diuretic therapy, diuretic infusions, intravenous (IV) dopamine, adenosine receptor antagonists, and vasopressin receptor antagonists.^{2–6} More recently, nesiritide, a recombinant human B-type natriuretic peptide (hBNP), has been proposed as an agent that may improve loop diuretic effect.⁷ The premise for this empiric use of nesiritide as adjunctive diuretic therapy includes its demonstrated vasodilatory and neuroinhibitory effects,

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with the latter being marked by reductions in both plasma aldosterone and plasma renin activity.^{8–10}

However, the impact of combination therapy with furosemide and nesiritide on urine output and neurohormonal activation in humans with heart failure (HF) has yet to be rigorously investigated in a randomized clinical trial. We sought to systematically compare the effects of nesiritide, furosemide, and their combination on natriuresis, diuresis, and plasma aldosterone levels in relatively diuretic-resistant patients with chronic stable HF who required moderately high doses of furosemide to sustain a clinically euvolemic state.³

Methods

Study Design

Scios Protocol 704.351 was an industry-sponsored, multicenter, randomized, open-label, three-arm crossover study conducted in a clinical research center setting. The protocol and informed consent form were approved by the institutional review boards of all participating institutions and adhered to appropriate standards for protection of human subjects. All participants provided written informed consent. Subjects had chronic symptomatic HF, defined as New York Heart Association class II or III with a left ventricular ejection fraction <40% within 6 months of study entry. Subjects were euvolemic or mildly volume overloaded, and clinically stable for at least 90 days prior to enrollment. Subjects were required to have received a medical regimen for HF, including angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), and/or β -blockers, without dose changes for at least 60 days. The study protocol defined “diuretic resistant” as requiring a daily dose of 80 to 240 mg oral furosemide for at least 7 days prior to the study.

Pertinent exclusion criteria included systolic blood pressure consistently <90 mm Hg; serum Na^+ <128 mEq/L; creatinine clearance <45 mL/min; changing renal function within 7 days of study enrollment; myocardial infarction within 90 days, unstable angina within 14 days, or any clinical evidence of active myocardial ischemia; recent HF decompensation within 30 days; or use of spironolactone, amiloride, eplerenone, or sildenafil within 5 days.

The study design required each subject to receive each of the 3 following interventions: (1) furosemide only, 40 mg IV bolus given over 2 minutes (the F treatment limb); (2) nesiritide alone, 2 $\mu\text{g}/\text{kg}$ IV bolus followed by a 0.01 $\mu\text{g}/\text{kg}/\text{min}$ continuous infusion lasting for 6 hours (the Nes treatment limb); (3) furosemide and nesiritide combined, given as nesiritide 2 $\mu\text{g}/\text{kg}$ IV bolus followed by a 0.01 $\mu\text{g}/\text{kg}/\text{min}$ continuous infusion lasting for 6 hours and a 40 mg IV bolus of furosemide given over 2 minutes ~15 minutes after initiation of the nesiritide infusion (the F/Nes treatment limb). These 3 interventions were administered in 3 study periods using an orthogonal Latin-square crossover

study design containing 6 unique treatment sequences. This crossover design was balanced in that each treatment was to be provided the same number of times in each treatment period, and each treatment followed every other treatment the same number of times. Each subject was randomly assigned to 1 of the 6 different intervention sequences.

Subjects remained in their respective clinical research centers for the entire 7 days of the study. The randomized treatments were administered on days 2, 4, and 6. Days 1, 3, and 5 were equilibrium or rest days, and subjects were discharged on day 7. Subjects were placed on a 100 mEq/day Na^+ and 80 mEq/day potassium (K^+) diet from 72 hours before study start until discharge from their respective study units. Free access to water was allowed during the off-treatment days of these studies. Oral fluid intake was limited to a maximum of 250 mL from the start of a treatment to 6 hours after. Net urine losses were replaced intravenously with 0.45% saline during the first 6 hours of a treatment day. Because of this liberal fluid repletion, serial BUN and serum creatinine determinations were not performed. All oral maintenance medications were continued unchanged during the study except for diuretics (including spironolactone), nonsteroidal anti-inflammatory drugs, cyclooxygenase-2 inhibitors, and sildenafil. Caffeine intake, including coffee, tea, chocolate, and caffeinated sodas, was not allowed while the subjects were housed. Safety was assessed continuously throughout the treatment phase and by telephone 7 to 14 days after discharge.

Laboratory Analyses

Urine Na^+ and K^+ were measured by flame photometry (IL-943; Instrumentation Laboratory, Lexington, MA). Plasma aldosterone (Diagnostic Products Corporation, Los Angeles, CA) and urinary cyclic guanosine monophosphate (cGMP; Amersham Life Science, Arlington Heights, IL) were measured by established and validated radioimmunoassay methods. Urinary furosemide was determined by a previously developed high-performance liquid chromatography method.

Statistical Analysis

Efficacy endpoints included urinary Na^+ and furosemide excretion, urinary flow rate, urinary excretion of cGMP, and the time-wise change from baseline in plasma aldosterone concentrations. Endpoint measures were analyzed using a 3-period, 3-treatment crossover analysis of covariance (ANCOVA) mixed model that included fixed effect parameters for sequence, treatment, and study period, with the baseline value as a covariate. A random effects term was included in the model for subjects nested within sequence. The primary efficacy measure was the excretion rate of urine Na^+ . The primary efficacy analysis was the difference between the F vs F/Nes treatment limbs. The study analysis also included a check for differential carryover

effects. Descriptive statistics were calculated for continuous endpoints and are presented as mean $\pm$ SE unless otherwise indicated. Proportions are presented for categorical and ordinal variables. *P* values <0.05 were considered significant. Analyses were performed using version 8.2 of the SAS software (SAS Institute, Inc., Cary, NC).

Results

Of the 42 subjects enrolled in the study, 2 withdrew consent before receiving study medication, 4 were discontinued because of adverse events, 1 was discontinued because of a protocol violation, and 7 (5 potential furosemide dosing errors, 1 nesiritide dosing error, and 1 suspected dietary noncompliance) did not have evaluable information. The remaining 28 subjects constitute the evaluable population. The demographic and clinical characteristics at baseline for these 28 subjects are presented in Table 1.

Concomitant medication use was identical for anti-diabetic agents, warfarin, and calcium channel blockers in each of the 3 treatment limbs. Eighteen subjects (64%) remained on an ACE inhibitor through all 3 treatment phases. ARB therapy differed slightly among the treatment groups (F: *n* = 11/28 [39%], Nes: *n* = 11/28 [39%], F/Nes: *n* = 9/28 [32%]).

The primary efficacy endpoint was the baseline-adjusted difference between F vs F/Nes with respect to the rate of urinary Na⁺ excretion. In that regard, there were no statistically significant combination therapy treatment effects at any time point (Figure 1A).

Urinary flow rate, as well as urinary excretion of Na⁺, cGMP, and furosemide, are summarized in Figure 1. The urinary excretion of Na⁺ and total urine output were similar at all time points for F compared with F/Nes (Figures 1A and 1B). Nes produced significantly less urinary excretion of Na⁺ and urine output during the initial 4 hours, but significantly greater urine output during hours 10–22 (Table 2). The urinary excretion of K⁺ showed no significant difference over the 0–4- and 10–22-hour intervals. Only the 4–10-hour interval showed a significant difference (*P* < 0.05) between F and F/Nes. The urinary excretion of cGMP, confirming the pharmacologic activity of nesiritide, increased significantly with nesiritide with or without furosemide, compared with furosemide alone (Figure 1C). The urinary excretion of furosemide in the 0–4-hour time frame was significantly lower when coadministered with nesiritide (Figure 1D). Serum potassium and creatinine values were obtained periodically during the hospitalization phase of these studies (and not at the time of active treatment) and did not show any systematic change.

At the 2-hour postdose time point, plasma aldosterone levels increased from baseline by 2.2 ± 1.6 (ng/dL) after F, decreased by 3.9 ± 1.6 (ng/dL) after Nes (*P* = 0.005 vs F and *P* = 0.563 vs F/Nes), and decreased by 2.8 ± 1.6 (ng/dL) for F/Nes (*P* = 0.023 vs F; Figure 2).

Table 1. Demographic and Clinical Characteristics at Baseline

	Evaluable Subjects (n = 28)
Age, y (mean $\pm$ SD)	54 $\pm$ 13
M, n (%)	23 (82)
Caucasian, n (%)	19 (68)
Weight, kg (mean $\pm$ SD)	106 $\pm$ 33
NYHA class, n (%)	
II	13 (46%)
III	15 (54%)
Primary HF etiology, n (%)	
Ischemic	13 (46)
Idiopathic, dilated cardiomyopathy	8 (29)
Hypertensive	4 (14)
Valvular or rheumatic heart disease	1 (4)
Other	2 (7)
Cardiac history, n (%)	
CAD	24 (86)
CABG or coronary angioplasty	13 (46)
Previous MI	16 (57)
Diabetes	10 (36)
Pacemaker/implantable defibrillator	8 (29)/5 (18)
Atrial arrhythmia/ventricular arrhythmia	4 (14)/6 (21)
Prior medications, n (%)	
Oral diuretics (per protocol)	28 (100)
ACE inhibitors/ARBs	19 (68)/9 (32)
β -Blockers/calcium channel blockers	19 (68)/2 (7)
Aspirin/clopidogrel	18 (64)/4 (24)
Digoxin	16 (57)
Statins	16 (57)
Aldosterone receptor antagonists ^a	14 (50)
Oral nitrates	10 (36)
Antidiabetic agents	10 (36)
Warfarin	7 (25)
Class III antiarrhythmics	2 (7)

Table 1. (continued)

	Evaluable Subjects (n = 28)
Prestudy oral furosemide dose in mg, median (range)	80 (80–320)
LVEF, % (mean ± SD)	26 ± 12
Systolic BP, n (%)	
≤100 mmHg	5 (18)
>100 mmHg and <140 mmHg	20 (71)
≥140 mmHg	3 (11)
BNP, pg/mL, median (range)	80 (5–5000)
Weight ≤115 kg (n = 19 [68%])	132 (25–2870)
Weight >115 kg (n = 9 [32%])	17 (5–5000)

Abbreviations: ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; BNP, B-type natriuretic peptide; BP, blood pressure; CABG, coronary artery bypass graft; CAD, coronary artery disease; HF, heart failure; n, number of patients; LVEF, left ventricular ejection fraction; M, male; MI, myocardial infarction; NYHA, New York Heart Association; SD, standard deviation.

^a Discontinued ≥5 days before study start.

Safety

During the course of the study, most subjects (28/40, 70.0%) experienced at least 1 adverse event (Table 3). Two of these adverse events were classified as serious, of which only 1 (symptomatic hypotension in a subject who erroneously received an excessive dose of nesiritide) was thought to be possibly related to the study drug.

Discussion

Our data show that there was no significant difference in urinary Na⁺ excretion between F and F/Nes, although there was a lesser amount of furosemide excreted during the 0–4-hour interval of the F/Nes treatment limb. This suggests that the intratubular furosemide concentrations achieved in the F/Nes treatment limb worked in a relatively more efficient manner. Our data also show that a nesiritide infusion initiated prior to IV loop diuretic administration inhibited the early (2- and 4-h) rise in aldosterone levels that occurred in response to IV furosemide alone (Figure 2 and 3). Urinary cGMP levels tracked nesiritide administration, suggesting that the observed suppression of aldosterone was indeed a pharmacologic effect of nesiritide.

A thorough understanding of the determinants of diuretic response is a prerequisite for the proper management of any diuretic-resistant state. In order to characterize

Figure 1. Urinary sodium excretion (A), urine output (B), urinary cyclic guanosine monophosphate (cGMP) excretion (C), and urinary furosemide excretion (D). * $P < 0.05$ nesiritide vs furosemide. † $P < 0.05$ nesiritide vs furosemide plus nesiritide. ‡ $P < 0.05$ furosemide vs furosemide plus nesiritide.

Table 2. Urine Na⁺ and K⁺ Excretion and Urine Output

	Nesiritide	Furosemide	Both
Urine Na ⁺ excretion rate (μmol/min), mean ± SE			
Predose	95.3 ± 15.4	118.7 ± 17.2	103.1 ± 15.5
0–4 h postdose	117.6 ± 15.1	591.1 ± 55.6 ^a	514.9 ± 62.1 ^a
4–10 h postdose	72.5 ± 6.8	80.1 ± 9.7	72.8 ± 8.8
10–22 h postdose	36.5 ± 5.4	34.6 ± 3.6	25.7 ± 4.5
Urine K ⁺ excretion rate (μmol/min), mean ± SE			
Predose	66.6 ± 8.35	64.1 ± 7.79	60.3 ± 7.82
0–4 h postdose	58.9 ± 5.90	120 ± 10.69	101.3 ± 13.78
4–10 h postdose	47.7 ± 4.42	56.2 ± 5.53 ^b	46.3 ± 4.32
10–22 h postdose	37.3 ± 4.79	32.8 ± 3.01	38.1 ± 5.37
Urine flow rate (mL/min), mean ± SE			
Predose	2.3 ± 0.4	2.5 ± 0.3	2.3 ± 0.3
0–4 h postdose	2.1 ± 0.2	6.4 ± 0.5 ^a	6.1 ± 0.6 ^a
4–10 h postdose	1.3 ± 0.1	1.4 ± 0.1	1.2 ± 0.1
10–22 h postdose	1.6 ± 0.3	1.2 ± 0.1 ^c	1.2 ± 0.2 ^c

Abbreviations: K⁺, potassium; Na⁺, sodium; SE, standard error.
^a $P < 0.001$ nesiritide.
^b $P < 0.05$ furosemide vs both.
^c $P < 0.05$ nesiritide.

the relationship between a dose of a loop diuretic (such as furosemide) and any ensuing natriuretic response, it is necessary to establish the pattern of loop diuretic urinary delivery.¹¹ As such, this measure was a component of these particular studies. Loop diuretics reach their luminal (or urinary) site of action through active secretion from blood into urine via proximal tubular organic anion transporters.^{12,13} A number of factors influence this secretion, including the amount of circulating drug, renal blood flow, systemic blood pressure, plasma-protein binding capacity, as well as the intrinsic tubular capacity for organic anion secretion.¹³ Once urinary drug delivery has occurred, the pharmacodynamics of the response emerge as the final determinant of the level of natriuresis.^{1,13}

Many patients with ADHF have distinctly abnormal diuretic dose–response relationships, such that very large doses (oral or IV) of a loop diuretic elicit little response.¹ Under these circumstances, diuretic-enhancing maneuvers are generally considered. A number of these maneuvers have been tried in patients with ADHF, including combination diuretic therapy, diuretic infusions, IV dopamine, adenosine-receptor antagonists, and vasopressin-receptor

Figure 2. Plasma aldosterone levels during the initial 6-hour treatment period. * $P < 0.001$ furosemide vs nesiritide and $P < 0.01$ furosemide plus nesiritide.

Table 3. Adverse Events During the Treatment Period^a

Event, n (%)	Furosemide (n = 36)	Nesiritide (n = 40)	Both (n = 36)	Overall (n = 40)
Any	8 (22.2)	16 (40)	17 (42.5)	28 (70)
Headache	2 (5.6)	1 (2.5)	3 (8.3)	5 (12.5)
Dizziness	1 (2.8)	3 (7.5)	3 (8.3)	6 (15)
Dizziness, postural	0 (0)	0 (0)	2 (5.6)	2 (5.0)
Hypotension	1 (2.8)	5 (12.5)	4 (11.1)	7 (17.5)
Asymptomatic	1 (2.8)	3 (7.5)	3 (8.3)	7 (17.5)
Symptomatic	0	2 (5.0)	1 (2.8)	3 (7.5)
Nausea	1 (2.8)	3 (7.5)	1 (2.8)	5 (12.5)
Rigors	0 (0)	2 (5.0)	0 (0)	2 (5.0)

Abbreviations: n, number of patients.

^a Defined as the start of study drug until the end of the washout period on the following day, ie, days 2–3, 4–5, and 6–7.

antagonists.^{2–6} More recently, nesiritide has been suggested to enhance the effect of loop-diuretic therapy relating to its generally favorable effect on cardiac hemodynamics, as well as by its positive effects on the neurohormonal profile that is typical of HF.^{7–10}

In this study, blood pressure measurements were followed from a safety perspective and were not obtained frequently enough to allow indexing of Na⁺ and furosemide excretion by change in blood pressure. However, there was a sufficient number of blood pressure readings to suggest that blood pressure decreased with nesiritide whether given alone or administered with furosemide. This reduction in blood pressure is the likely explanation for the early-phase

Figure 3. Maximum change from baseline in plasma aldosterone after furosemide, nesiritide, and the combination of both furosemide and nesiritide.

reduction in the tubular delivery of furosemide in the F/Nes treatment limb.

Enhanced neurohormonal activation in response to diuretics remains an important consideration in the sustained effectiveness of diuretic therapy in HF.^{3,14} Neurohormonal activation due to loop diuretics can occur in a matter of minutes as a consequence of a direct stimulatory effect on the renin-angiotensin-aldosterone system (RAAS), independent of volume change.¹⁵ This early activation of the RAAS may diminish diuretic responsiveness. Loop diuretics also produce subacute and chronic RAAS activation. This process serves as one of the confounding factors behind relative diuretic resistance in HF.^{3,11,16,17} The demonstration that concomitant use of nesiritide with furosemide blocked the aldosterone increase seen with furosemide alone suggests that coadministration of these drugs may preempt the neurohormonal contribution to the blunted diuretic response present during the volume-mobilization stage of acute HF management. Confirmation of this hypothesis will require further studies.

Study Limitations

There are several limitations to this study. First, there was a high dropout rate in the study, largely due to the complexity of the protocol. Second, based on the protocol requirements and supported by the BNP levels, patients in this study did not have evidence of significant volume overload; these patients were clinically stable, not acutely decompensated. Therefore, assessing the clinical significance of these data and applying the results to patients with symptomatic ADHF will require additional confirmatory investigations. Finally, despite the fact that our subjects required an 80-mg median maintenance dose of furosemide at baseline, these patients were not severely “diuretic resistant,” as defined by many clinicians. However, their urinary sodium excretion rates were much lower

than what would be expected if the same dose of 4051 IV furosemide were given to normal subjects. Because of this, we have employed the qualifier “relatively diuretic resistant” to describe their clinical status.

Conclusion

In clinically stable HF subjects with relative diuretic resistance, the observed natriuretic/diuretic response to nesiritide and furosemide combined was similar to furosemide alone; nesiritide alone did not result in significant diuresis. Although nesiritide/furosemide combined and furosemide alone resulted in similar Na^+ and water excretion, furosemide alone differentially resulted in a prompt rise in plasma aldosterone. Coadministration of nesiritide with furosemide prevented this neurohormonal response. The clinical significance of aldosterone suppression with the combination of nesiritide and furosemide will require further study. However, this interaction may be important in patients with ADHF, where neurohormonal activation plays an important role in shaping diuretic responsiveness.

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