Renal and Hormonal Effects of Systemic Nitric Oxide Inhibition in Patients With Congestive Heart Failure and in Healthy Control Subjects

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ABSTRACT

Background: The significance of basal renal nitric oxide (NO) availability in the regulation of renal perfusion and sodium excretion in human congestive heart failure (CHF) has not been described previously.

Methods and Results: We studied the effects of acute systemic NO synthesis inhibition with N^G-monomethyl-L-arginine (L-NMMA) in 12 patients with CHF and 10 healthy control subjects (CON) in a randomized placebo-controlled study. Effect parameters were renal plasma flow (RPF), renal vascular resistance (RVR), glomerular filtration rate (GFR), urine sodium excretion and plasma levels of vasoactive hormones. L-NMMA was associated with a significant decrease in RPF (CON-LNMMA: $-13 \pm 3\%$ [$P = .014$]; CHF-LNMMA: $-17 \pm 7\%$ [$P = .017$]) and a profound increase in RVR in both CHF and CON (CON-LNMMA: $+26 \pm 6\%$ [$P = .009$]; CHF-LNMMA: $+37 \pm 70\%$ [$P = .005$]). Significant decreases in sodium excretion were found in both CHF-LNMMA and CON-LNMMA. Relative changes from baseline were not statistically different between CHF-LNMMA and CON-LNMMA. After L-NMMA, RPF values correlated inversely with plasma aldosterone in CHF-LNMMA ($P = .01$). L-NMMA induced an increase in A-type natriuretic peptide (ANP) only in CHF-LNMMA ($+18 \pm 8\%$; $P = .035$), which correlated significantly with basal ANP levels ($P = .034$).

Conclusions: There was no difference in the renal response to L-NMMA in CHF vs CON, suggesting that the impact of NO on renal perfusion and sodium excretion is maintained in stable CHF. We suggest that NO influences the release of ANP during high levels of atrial stretch in CHF. (*J Cardiac Fail 2013;19:776–785*)

Key Words: Nitric oxide, N^G-monomethyl-L-arginine, renal plasma flow, ANP, aldosterone.

Congestive heart failure (CHF) is generally accompanied by increased peripheral resistance and increased sodium and water retention by the kidneys. Patients with CHF usually develop a disproportionate decrease in renal blood flow and glomerular filtration rate (GFR), leading to increased renal vascular resistance (RVR) and increased filtration fraction.[1] Renal failure may develop due to severely compromised tissue perfusion and intense neurohormonal activation, including increased activity of the renin-angiotensin system, endothelin-1, and the sympathetic nervous system. The presence of severe sodium and water retention and overt renal failure during CHF is associated with a poor prognosis regarding survival.[2] Nitric oxide (NO) is an important endothelium-derived vasodilator which exerts a continuous vasodilating tone in the systemic and renal circulations.[3] In the kidneys, NO is a strong promoter of natriuresis by acting on a number of natriuretic mechanisms.[3] The activity and relative influence of the L-arginine (L-arg)–NO pathway during CHF has therefore attracted considerable interest, because decreased NO availability could be of pathophysiologic significance to the vascular and renal derangements observed during CHF.

A large number of studies of the peripheral vasculature in animals and humans have demonstrated impaired endothelial function during CHF thought to be caused by decreased NO availability.[4,5] However, other studies are in disagreement with this.[6] Regarding renal NO activity during CHF,

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See page 784 for disclosure information.

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few human studies are available. It has been demonstrated that renal perfusion and sodium excretion can be stimulated by L-arg infusion in patients with CHF or by intrarenal infusion of acetylcholine (Ach), suggesting that renal NO availability in human CHF is not exhausted.[7,8] No human studies are available regarding the basal (unstimulated) renal NO activity. This issue can be further elucidated by studying the renal effects of NO inhibition in patients with CHF.

The aims of the present study were:

(1) To study the effects of acute systemic treatment with N^G-monomethyl-L-arginine (L-NMMA), which is an unselective competitive inhibitor of NO-synthase, on renal hemodynamics (renal plasma flow (RPF), GFR, renal sodium excretion, renal tubular sodium handling (fractional lithium excretion) and plasma levels of A-type natriuretic peptide (ANP), B-type natriuretic peptide (BNP), guanosine 3′,5′-cyclic monophosphate (P-cGMP), renin, angiotensin II (ANGII), and aldosterone (ALDO).
(2) To compare these results with acute effects of L-NMMA in a group of healthy control subjects.

We hypothesized that stable CHF in humans is associated with reduced NO availability and reduced renal response to NO inhibition with L-NMMA.

Methods

Patients and Study Design

The study was approved by the local Ethics Committee and the Danish National Board of Health. Informed consent was obtained from each of the participating subjects. The study was randomized, placebo controlled, and single blinded regarding L-NMMA/placebo. We included 2 groups of patients with CHF, 1 group was allocated to 1 experiment with acute L-NMMA treatment (CHF-LNMMA; n = 12) and 1 group was allocated to 1 experiment with placebo (CHF-Placebo; n = 10). A group of healthy control subjects (CON; n = 10) was studied twice in a cross-over fashion during either L-NMMA (CON-LNMMA) or placebo (CON-Placebo) experiment in randomized order. At least 14 days separated the cross-over experiments. For ethical reasons we did not apply the cross-over design to the CHF patients, because of the preexperimental phase where important medications had to be withdrawn.

CHF patients were recruited according to the following criteria:

(1) Chronic CHF with a ventricular ejection fraction (EF) of <0.40 and a clinical condition corresponding to New York Heart Association (NYHA) functional class II−III. Diagnosis was made by relevant history and echocardiography and/or radionuclide ventriculography.
(2) Age 20−70 years.
(3) Stable clinical condition defined as no significant change in medication for 6 weeks.

Exclusion criteria were: (1) valve disease with stenosis; (2) severe arrhythmia; (3) recent (<3 months) myocardial infarction or coronary by-pass surgery; (4) diseases of the liver, kidneys, endocrine organs, or lungs judged to be not secondary to CHF; (5) history of cancer or cerebrovascular insult; (6) severe hypertension.

Control subjects were required to have no history of disease of the heart, liver, kidneys, or endocrine organs, no history of alcohol or drug abuse, and no current medical treatment. Before the study, all participants had a clinical examination, blood pressure measurement, electrocardiogram and laboratory screening, including hematology, P-electrolytes, P-creatinine, B-glucose, P-cholesterol, P-bilirubin, P-alkaline phosphatase, P-alanine aminotransferase, and prothrombin time. Urine was analyzed by dipstick. The results of these tests were required to be normal before inclusion.

Medication

During the study, medication in CHF patients was handled according to the following directions. Treatment with digitalis, β-blocking agents, and calcium antagonists were not discontinued. Angiotensin-converting enzyme inhibitors or angiotensin II receptor antagonists or long-acting nitrates were discontinued 1 week before the study. Short-acting nitrates were discontinued ≥6 hours before the experiment. Diuretics were discontinued on the day of the experiment and resumed immediately after the experiment.

Clearance Experiments

All procedures and measurements were identical during L-NMMA and placebo experiments. All subjects performed 24-hour urine collections the day before each experiment. Tablet lithium carbonate (300 mg) was ingested at 22:00 the night before for the measurement of lithium clearance. On the experiment day, an oral water load of 200 mL tap water every 30 minutes was started at 07:00. Two indwelling catheters for blood sampling and administration of tracers and study drugs were placed in forearm veins, one in each arm. Urine was collected by voiding in the standing or sitting position and the subjects were otherwise kept in the supine position. At 08:30, priming doses of ^{51}Cr-EDTA and ^{125}I-hippuran were given, followed by sustained infusions of both. After an equilibration time of 60 minutes, the study continued with clearance periods of 30 minutes. Three baseline periods were obtained from 09:30 to 11:00. At 11:00, bolus injection of either L-NMMA (3 mg/kg) or placebo was given over 10 minutes. The study continued with 4 clearance periods to evaluate the effects of treatment. Blood samples were drawn every 30 minutes, ie, before the first clearance period and at the end of each period and were analyzed for tracers, standard electrolytes, lithium, and osmolality. In addition, analysis of the plasma levels of cGMP, renin, ANGII, ALDO, ANP, and BNP were performed from blood samples withdrawn at 11:00 (baseline), 12:00 (60 minutes after treatment), and 13:00 (120 minutes after treatment, end of experiment). Urine collections were analyzed for tracers, standard electrolytes, lithium, osmolality, and cGMP (U-cGMP).

GFR and RPF were measured by the constant infusion clearance technique using ^{51}Cr-EDTA and ^{125}I-hippuran, respectively, as reference substances. A priming dose ensured a serum activity of 200−600 cpm/mL for ^{51}Cr-EDTA and 800-1,600 cpm/mL for ^{125}I-hippuran. Serum activity was kept stable by constant intravenous infusion with a volume-controlled infusion pump.

Blood pressure was determined by a semiautomatic oscillometric device. Blood pressures were measured every 30 minutes except after L-NMMA/placebo treatment, when pressures were measured every 5 minutes for a 30-minute period.