Bradykinin Contributes to the Systemic Hemodynamic Effects of Chronic Angiotensin-Converting Enzyme Inhibition in Patients With Heart Failure

Nicholas L.M. Cruden, Fraser N. Witherow, David J. Webb, Keith A.A. Fox, David E. Newby

Background—Bradykinin is an endogenous vasodilator that may contribute to the systemic effects of angiotensin-converting enzyme (ACE) inhibitor therapy. Using B9340, a bradykinin receptor antagonist, we determined the contribution of bradykinin to the systemic hemodynamic effects of long-term ACE inhibition in patients with chronic heart failure.

Methods and Results—Fourteen patients with heart failure received enalapril (10 mg twice daily) or losartan (50 mg twice daily) in a randomized double-blind crossover trial. After 6 weeks treatment, patients underwent right heart catheterization and were randomized to an intravenous infusion of B9340 (2 to 20 μg/kg per minute) or saline placebo. After B9340 infusion in patients treated with enalapril, mean arterial pressure (+5.2 mm Hg), systemic vascular resistance (+315 dynes−s/cm⁵), pulmonary arterial wedge pressure (−1.4 mm Hg), and mean pulmonary arterial pressure (−1.3 mm Hg) were greater compared with losartan (P<0.005, P=0.07, P<0.0001, and P<0.05 respectively) or placebo infusion (P≤0.005 for all). There was a reduction in cardiac output after B9340 with enalapril compared with placebo (P<0.001) but not losartan.

Conclusions—Bradykinin contributes to the systemic hemodynamic effects of long-term ACE inhibition in patients with heart failure. This mechanism may explain the apparent clinical differences between ACE inhibitors and angiotensin receptor blockers in the treatment of heart failure. (Arterioscler Thromb Vasc Biol. 2004;24:1043-1048.)

Key Words: ACE inhibitors • bradykinin • heart failure • hemodynamics • pharmacology

Activation of the renin-angiotensin-aldosterone system plays a key role in the pathogenesis of chronic heart failure, leading to salt and water retention and peripheral vasoconstriction. Inhibitors of angiotensin-converting enzyme (ACE) reduce peripheral vascular tone and systemic arterial pressure and improve symptoms and survival in patients with chronic heart failure. Although previously attributed to a reduction in angiotensin-mediated vasoconstriction, recent data have indicated that the hemodynamic changes associated with ACE inhibitor therapy may be caused, at least in part, by the inhibition of bradykinin metabolism.

The bradykinin metabolizing properties of ACE (kininase II) were first described in 1967. Indeed, ACE has greater affinity for bradykinin than for angiotensin I and is the principal enzyme responsible for the rapid turnover of bradykinin (plasma half life ≈15 seconds). This rapid metabolism makes accurate measurement of bradykinin difficult, although elevated plasma bradykinin concentrations have been reported in the presence of ACE inhibition. Bradykinin is a powerful endothelium-dependent vasodilator and, at a functional level, inhibition of ACE potentiates the action of exogenous bradykinin in the human forearm and coronary circulations.

There are 2 principal kinin receptor subtypes in humans, B₁ and B₂. The endothelial B₂ receptor is constitutively expressed and mediates the vasodilator and profibrinolytic effects of bradykinin. The vascular B₁ receptor is normally expressed very weakly but is markedly upregulated in the presence of inflammation, cardiovascular disease states and ACE inhibition, where it also mediates vasodilatation.

The selective kinin receptor antagonists, HOE-140 (icatibant) and B9340, are synthetic peptide analogues of bradykinin with different inhibitory activities at the specific kinin receptor subtypes. HOE-140 acts solely at the B₂ receptor, whereas B9340 blocks kinin activity mediated via both B₁ and B₂ receptors. Several investigators have reported that intravenous HOE-140 attenuates the vasodepressor response associated with acute ACE inhibition, both in healthy volunteers and in hypertensive patients. In contrast, B9340, but not HOE-140, induced vasoconstriction in the forearm circulation of patients with heart failure treated with chronic ACE inhibitor therapy. Besides implicating bradykinin in the hemodynamic changes associated with ACE inhibition, these
data raise the possibility of a role for the B\textsubscript{1} receptor in patients with chronic heart failure.\textsuperscript{18}

The aims of the present study were to demonstrate that the kinin receptor antagonist, B9340, inhibits bradykinin activity in the systemic circulation and to determine whether endogenous bradykinin contributes to the hemodynamic effects of long-term ACE inhibition in patients with chronic symptomatic heart failure.

Methods

Subjects and Patients
The protocols were performed with the approval of the local research ethics committee and in accordance with the Declaration of Helsinki (2000). Written informed consent was obtained from each subject.

Six healthy, male nonsmokers attended fasted at 8:00 AM on 2 occasions at least 1 week apart. Volunteers avoided alcohol and caffeine for 24 hours before each study and received no medications or vasoactive substance in the preceding seven days.

Fourteen patients with stable New York Heart Association (NYHA) class II–III chronic heart failure and objective evidence of left ventricular impairment (left ventricular ejection fraction <40%, shortening fraction <20%, or left ventricular end-diastolic diameter >5.5 cm) were enrolled into the study. Patients were included only if they had been established on maximally tolerated ACE inhibitor therapy for at least 6 months. Patients were excluded if they had significant valvular heart disease, renal or hepatic failure, or had previous malignant ventricular arrhythmias. After initial screening, patients received enalapril 10 mg\textsuperscript{2} or losartan 50 mg\textsuperscript{20} both twice daily, in place of their usual ACE inhibitor in a randomized, double-blind crossover trial. After 6 weeks of each treatment, subjects attended fasted at 8:00 AM and underwent right heart catheterization. On the morning of each visit, oral study medication was administered at 7:00 AM to achieve peak plasma concentrations of the active metabolites, enalaprilat or E-3174, respectively, during B9340 or placebo infusion (10:00 to 11:00 AM). Diuretics were withheld on the morning of each study for patient comfort.

Measurements

Healthy Volunteer Study
Intra-arterial drug administration and forearm blood flow measurements were performed using venous occlusion plethysmography as previously described.\textsuperscript{18} After each forearm blood flow measurement, heart rate and blood pressure were determined noninvasively in the noninfused arm using a semiautomated, sphygmomanometer (UA-731; A&D Engineering, Milpitas, Calif).

Patient Study
A 9-French venous sheath was inserted aseptically under local anesthesia via the right femoral vein. Under fluoroscopic screening, a continuous cardiac output thermodilution Swan-Ganz catheter (Edwards Lifesciences, Irvine, Calif) was positioned in the pulmonary arterial tree. Pulmonary arterial pressure, pulmonary arterial wedge pressure, and central venous pressure were recorded using a Hewlett Packard monitor (U78339A; Hewlett Packard, Andover, Mass). Continuous cardiac output was recorded using a Vigilance monitor (Edwards Lifesciences, Irvine, Calif). Heart rate and blood pressure were measured noninvasively using a semiautomated sphygmomanometer (U78339A; Hewlett Packard, Andover, Mass).

Drugs
B9340 (molecular weight 1318.6) is a synthetic peptide antagonist of bradykinin with potent inhibitory activity at both the B\textsubscript{1} and B\textsubscript{2} receptors (pIC\textsubscript{50} in vitro of 8.1 and 9.8, respectively).\textsuperscript{10} The doses of B9340 and bradykinin were chosen after the results of dose ranging studies performed in the forearm circulation of healthy volunteers.\textsuperscript{21} Pharmaceutical grade B9340 and bradykinin were supplied by Clinafa AG (Läufelfingen, Switzerland) and dissolved in saline on the day of study.

Protocol Design

Healthy Volunteer Study
After 30 minutes equilibration with saline infusion, bradykinin was infused via the brachial artery of the nondominant arm at 300 pmol/min for 120 minutes. B9340 or saline placebo was infused intravenously in the contralateral arm at 2, 6, and 20 \(\mu\)g/kg per minute for 12 minutes at each dose with 12 minutes separating each dose. Forearm blood flow was measured at 6 minute intervals throughout each study.

Patient Study
After a 30 minute infusion of 50 mL saline, patients received an intravenous infusion of B9340 at 2, 6, and 20 \(\mu\)g/kg per minute for 15 minutes at each dose or saline placebo (75 mL) in a randomized, double-blind manner. The randomization was weighted such that 10 patients on enalapril received B9340, 10 patients on losartan received B9340, and 6 patients on either enalapril or losartan received placebo. Hemodynamic measurements were recorded at –40, –30, –10, 0 (baseline), +7, +15, +22, +30, +37, +45, +60, +75, +90, and +105 minutes during each study. Venous blood was collected at 0 (baseline), +45, and +105 minutes for determination of plasma ACE activity and plasma angiotensin II concentrations.

Laboratory Analysis
Blood samples were collected on ice, centrifuged immediately, and the resulting supernatant stored at –70°C until assayed. Plasma ACE activity was determined using colorimetric spectrophotometry (SIGMA Diagnostics, St Louis, Mo).\textsuperscript{22} After extraction using Bond Elut columns (Varian; Harbor City, Calif), plasma angiotensin II (Diasorin, Stillwater, Minn) concentrations were determined by radioimmunoassay.\textsuperscript{23}

Data Analysis and Statistics
Data are expressed as mean±standard error of the mean. Mean arterial pressure was defined as diastolic pressure plus one third of the pulse pressure. Forearm blood flows were calculated from plethysmographic data as described previously.\textsuperscript{18} In patient studies, hemodynamic parameters were assessed over time as absolute change from baseline (0 minutes) using 1-way analysis of variance (ANOVA) with repeated measures. Comparisons between treatment groups were made using 2-way ANOVA for which the within-subject variables were drug and time. Plasma neurohormone concentrations were compared at baseline using an unpaired \(t\) test. Statistical significance was taken at the 5% level.

Results

Healthy Volunteer Study
Bradykinin caused a sustained increase in forearm blood flow during placebo coinfusion (\(P<0.0001\); Figure 1). Systemic infusion of B9340 inhibited bradykinin-mediated vasodilatation in a dose-dependent manner (\(P<0.0001\); Figure 1).

Patient Study
Fourteen patients participated in the hemodynamic study. After the first visit, 1 patient withdrew because to worsening heart failure unrelated to treatment and was replaced. There were no significant differences in patient characteristics or baseline hemodynamic parameters between treatment groups (Table).

Plasma Neurohormones
Plasma ACE activity (13.2±2.4 versus 38.8±4.8 U/L; \(P<0.0001\)) and plasma angiotensin II concentrations (3.5±0.5 versus 12.3±2.3 pg/mL; \(P<0.005\)) at baseline on...
the morning of hemodynamic studies were significantly lower in patients treated with enalapril compared with losartan respectively. There were no significant changes in plasma ACE activity or angiotensin II concentrations during or after B9340 or placebo infusion (data on file).

**Mean Arterial Pressure and Heart Rate**

There were no significant changes in heart rate or mean arterial pressure during placebo infusion or after losartan therapy. Mean arterial pressure increased after administration of B9340 in patients treated with enalapril ($P<0.005$; Figure 2), although there was no significant change in heart rate. This pressor effect was greater in patients treated with enalapril than those given losartan therapy or placebo infusion ($P<0.005$ and $P<0.0001$, respectively; Figure 2).

**Cardiac Output and Systemic Vascular Resistance**

There were no significant changes in cardiac output or systemic vascular resistance during placebo infusion or after losartan therapy. In patients treated with enalapril receiving B9340, there was a trend toward an increase in systemic vascular resistance ($P=0.08$) but no change in cardiac output (Figure 2). Compared with placebo infusion, there was an increase in systemic vascular resistance and a fall in cardiac output after B9340 infusion in enalapril treated patients ($P<0.0005$ and $P<0.001$, respectively; Figure 2). There was a trend toward an increase in systemic vascular resistance in the enalapril treated group compared with those receiving losartan ($P=0.07$; Figure 2).

**Central Pressures**

Central venous pressure, pulmonary arterial wedge pressure, and mean pulmonary arterial pressure all decreased signifi-
cantly over time during placebo infusion \(P < 0.05\) for all; Figure 3). Compared with losartan therapy or placebo infusion, B9340 attenuated the decrease in pulmonary arterial wedge pressure \(P < 0.0001\) for both) and mean pulmonary arterial pressure \(P < 0.05\) and \(P = 0.005\), respectively) in patients treated with enalapril (Figure 3). There were no significant differences in the reductions of central venous pressure between treatment groups (Figure 3).

### Adverse Events

There were no major drug-related adverse events reported during healthy volunteer or patient studies. Three healthy volunteers and 1 patient reported mild self-limiting diarrhea after B9340 infusion.

### Discussion

We have demonstrated that intravenous B9340, a selective peptidic antagonist of bradykinin receptors, causes systemic inhibition of bradykinin-mediated vasodilatation in a dose-dependent manner. This bradykinin receptor antagonist has a significant pressor effect in patients with chronic heart failure maintained on long-term ACE inhibitor but not angiotensin receptor blocker therapy. These findings provide convincing evidence that bradykinin contributes to the hypotensive effects of chronic ACE inhibitor therapy in patients with heart failure.

We have previously shown that B9340 causes vasoconstriction in the forearm circulation in patients with heart failure treated with long-term ACE inhibitor therapy.\(^{18}\) The results of the present study confirm that these effects are important in the systemic circulation. This is consistent with published data demonstrating that the bradykinin receptor antagonist, HOE-140, attenuates the acute vasodepressor response to ACE inhibition. Gainer et al demonstrated that in salt-deplete healthy volunteers and hypertensive subjects, a systemic infusion of HOE-140 attenuated the hypotensive response to a single dose of captopril.\(^{16}\) The reduction in blood pressure observed after coadministration of HOE-140 and captopril was similar to that seen with the angiotensin receptor blocker, losartan.\(^{18}\) As the authors point out, previous studies have demonstrated that the peak hypotensive responses to captopril and losartan occur 1 and 4 hours, respectively, after drug administration.\(^{16}\) It has been suggested that these differences could account for the observed differences in the blood pressure response. To overcome this potential methodological difficulty, we chose to compare the ACE inhibitor, enalapril, at a dose previously shown to reduce mortality in patients with heart failure\(^2\) with the angiotensin receptor antagonist, losartan, administered at the maximum daily dose. Both these agents have long-acting active metabolites, enalaprilat and E-3174, respectively, achieving peak plasma concentrations 3 to 4 hours after ingestion that coincided with systemic intravenous administration of B9340 or placebo.\(^{24,25}\) It should also be recognized that we have assessed the effects of kinin receptor blockade in patients maintained on long-term ACE inhibitor therapy rather than the acute effects of a single oral dose. Moreover,
patients with heart failure maintained on chronic ACE inhibitor therapy may upregulate vascular B1 kinin receptor expression and we therefore used a combined B1 and B2 receptor antagonist to characterize more precisely the role of bradykinin.

It has been argued that angiotensin receptor blockade might potentially be more effective at blocking the detrimental effects of angiotensin II than ACE inhibition because in a substantial proportion of patients treated with chronic ACE inhibition, plasma angiotensin II concentrations increase over time returning toward pretreatment values. This “ACE escape” is thought to be caused by angiotensin II formation by non-ACE dependent pathways and may be associated with a poorer prognosis. However, clinical evidence that angiotensin receptor blockers have superior efficacy to ACE inhibitor therapy has proved elusive. In contrast, recent trials have confirmed that the therapeutic benefits of ACE inhibitors and angiotensin receptor blockers are additive: combination therapy improving symptoms and cardiovascular mortality in patients with heart failure when compared with ACE inhibition alone. Our findings may help to explain the additive benefits of combined ACE inhibition and angiotensin receptor blockade.

Large-scale clinical trials have demonstrated that ACE inhibitors not only improve survival but reduce the incidence of myocardial ischemia. This may be explained, in part, by the observation that ACE inhibition improves the fibrinolytic balance in patients with heart failure and ischemic heart disease. Bradykinin is not only a potent vasodilator but is intimately involved with the coagulation and fibrinolytic cascades. Indeed, it is a powerful mediator of endogenous fibrinolysis through the release of endothelium-derived tissue-type plasminogen activator. In patients with heart failure, chronic ACE inhibition markedly augments local bradykinin-mediated release of tissue-type plasminogen activator. Thus, potentiation of the other vascular actions of bradykinin may also contribute to the clinical benefits of ACE inhibitor therapy.

In patients treated with losartan, there was a small but significant increase in mean arterial pressure and systemic vascular resistance after B9340 infusion compared with placebo. This may have reflected the significant protein load associated with B9340 infusion and a protein-based placebo, such as albumin, may have been a more appropriate control. There are data indicating that bradykinin may contribute to the vascular effects of angiotensin receptor blockers. In transgenic mice overexpressing the AT1 receptor, angiotensin II causes vasodilatation that is attenuated by HOE-140 and in man, HOE-140 inhibits the improvement in flow-mediated vasodilatation associated with the angiotensin receptor blocker, candesartan. Finally, the findings are consistent with the possibility that bradykinin also contributes to the maintenance of blood pressure and vascular tone in patients with heart failure independently of ACE.

In summary, we have shown that in patients with chronic heart failure, systemic infusion of the combined kinin receptor antagonist, B9340, attenuates the vasodepressor effects associated with long-term enalapril therapy when compared with treatment with the angiotensin receptor blocker, losartan. We conclude that in patients with chronic symptomatic heart failure, bradykinin contributes to the systemic hemodynamic effects associated with long-term ACE inhibitor therapy.

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