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

Fraser N. Witherow, MB, ChB, MRCP; Ahmed Helmy, MB, ChB, PhD; David J. Webb, MD, FRCP, FRCPA, FFPM, FMed Sci; Keith A.A. Fox, BSc (Hons), MB, ChB, FRCP, FESC; David E. Newby, BSc (Hons), PhD, BM, DM, MRCP

Background—Bradykinin, an endogenous vasodilator peptide, is metabolized by ACE. The aims of the present study were to determine the doses of B9340, a bradykinin receptor antagonist, that inhibit vasodilatation to exogenous bradykinin and to assess the contribution of bradykinin to the maintenance of basal vascular tone in patients with heart failure receiving chronic ACE inhibitor therapy.

Methods and Results—Forearm blood flow was measured using bilateral venous occlusion plethysmography. On three occasions in a double-blind randomized manner, 8 healthy volunteers received intrabrachial infusions of placebo or B9340 (at 4.5 and 13.5 nmol/min). On each occasion, placebo or B9340 was coinfused with bradykinin (30 to 3000 pmol/min) and substance P (4 to 16 pmol/min). B9340 caused no change in basal FBF but produced dose-dependent inhibition of the vasodilatation to bradykinin ($P<0.001$) but not substance P. The effects of bradykinin antagonism were studied in 17 patients with NYHA grade II through IV heart failure maintained on chronic ACE inhibitor therapy. Incremental doses of B9340, but not HOE-140, produced a dose-dependent vasoconstriction ($P<0.01$). After withdrawal of ACE inhibitor therapy, B9340 produced no significant change in forearm blood flow. After reinstitution of therapy, B9340 again resulted in vasoconstriction ($P<0.03$).

Conclusions—B9340 is a potent and selective inhibitor of bradykinin-induced vasodilatation. Bradykinin does not contribute to the maintenance of basal peripheral arteriolar tone in healthy humans or patients with heart failure but contributes to the vasodilatation associated with chronic ACE inhibitor therapy in patients with heart failure via the $B_1$ receptor. (Circulation. 2001;104:2177-2181.)

Key Words: bradykinin $\blacklozenge$ heart failure $\blacklozenge$ blood flow

Patients with heart failure have a reduced cardiac reserve that is associated with neurohumoral activation of the renin-angiotensin-aldosterone system and peripheral vasoconstriction. Through blockade of angiotensin I conversion, ACE inhibitor therapy limits the generation of angiotensin II, thereby reducing the associated vasoconstriction and sodium and water retention. It has been widely established that ACE inhibitor therapy has major therapeutic benefits in patients with heart failure,1,2 which include improvements in morbidity, exercise capacity, and mortality.1,3 The administration of ACE inhibitor therapy in these patients causes systemic vasodilatation4 that has been attributed to the loss of angiotensin II–mediated vasoconstriction. However, it is unknown whether the vasodilatation associated with ACE inhibitor therapy may in part relate to the concomitant blockade of bradykinin degradation.

Bradykinin is a potent endothelium-dependent vasodilator5,6 that has a brief duration of action (plasma half-life of 15 to 30 sec) because of its rapid degradation by ACE. Indeed, ACE breaks down >95% of bradykinin in a single passage through the pulmonary circulation.7 Exogenous administration of bradykinin induces vasodilatation of epicardial coronary8,9 and resistance arteries in humans, which is mediated in part by nitric oxide10 and endothelium-derived hyperpolarizing factor.11 The local and systemic vascular effects of exogenous bradykinin administration can be enhanced by ACE inhibition.7,12 In hypertensive patients and sodium-deplete volunteers, Gainer et al13 have shown that systemic infusion of HOE-140 (icatibant), a highly selective bradykinin $B_2$ receptor antagonist, attenuated the hypotensive effects of captopril and resulted in a similar reduction in blood pressure to losartan, an angiotensin II type 1 receptor blocker.
This suggests that some of the short-term hypotensive effects of ACE inhibition are mediated by augmentation of endogenous bradykinin. This study has, however, been criticized for its design, because the observed pressor effect may have been attributable to the differing pharmacokinetic profiles of losartan and captopril. Moreover, it did not address other pertinent issues, such as the effect of chronic ACE inhibition or the potential role of the B1 receptor.

When examining in vivo vascular responses in humans, systemic drug administration causes concomitant effects on organs, such as the brain, kidney, and heart, and influences neurohumoral reflexes through changes in systemic hemodynamic parameters. Because of these influences, vascular responses cannot be wholly attributed to a direct effect of the drug on blood vessels. In contrast, the use of bilateral forearm blood flow measurements, with unilateral brachial artery infusion of vasoactive drugs at subsystemic, locally active doses, provides a powerful and reproducible method of directly assessing vascular responses in vivo. This technique has been used to demonstrate the major contribution of nitric oxide and endothelin-1 to the maintenance of basal peripheral vascular tone and to predict the pressor and depressor effects of systemic nitric oxide inhibition and endothelin receptor antagonism, respectively.

Several peptidic and nonpeptidic bradykinin receptor antagonists are presently under development. However, to date, there has been no assessment of the combined contribution of B1 and B2 receptors to basal vascular tone in patients with heart failure maintained on chronic ACE inhibitor therapy. B9340, a peptic analog of HOE-140, has inhibitory activity against both B1 and B2 receptors and has recently become available for use in clinical studies. Therefore, the aims of the present study were, first, to confirm that B9340 is a potent and specific bradykinin receptor antagonist in vivo in humans; second, to establish the role of bradykinin in the maintenance of peripheral vascular tone in healthy volunteers; and, third, to determine whether endogenous bradykinin contributes to the maintenance of basal peripheral vascular tone in patients with heart failure maintained on chronic ACE inhibitor therapy using both the selective and nonselective bradykinin receptor antagonists, HOE-140 and B9340, respectively.

Methods

Subjects and Patients

The protocols were performed with the approval of the local ethics committee in accordance with the Declaration of Helsinki (1989) and with the written informed consent of each subject. Eight healthy male nonsmokers attended on 3 separate occasions at least 1 week apart. Each volunteer was studied at the same time of day and was fasting for at least 4 hours and avoided alcohol and caffeine for 24 hours before each study. No medications or vasoactive drugs were taken in the 7 days before the study.

Seventeen patients with stable NYHA grade II through IV heart failure, established for at least 6 months on maximally tolerated ACE inhibitor therapy, were enrolled in the study. The patients attended fasting before each study, and diuretics were withheld on the morning of the study for patient comfort. Twelve patients initially attended at 9:00 AM on two occasions 1 week apart. ACE inhibitor therapy was withheld for 1 week before the second visit and recommenced after the second study. Nine patients returned at least 4 weeks after recommencing their ACE inhibitor therapy and underwent a repeat study. Two patients withdrew their consent because of personal reasons, and 1 patient changed therapy before the third visit and was thus excluded. Nine patients, including 4 patients who attended on the first three occasions, were subsequently studied on a final occasion.

Measurements

Intra-arterial drug administration and forearm blood flow measurements were performed as previously described. Heart rate and blood pressure were measured noninvasively in the noninfused arm immediately after the FBF measurements every 10 minutes throughout each study using a semiautomated, sphygmomanometer (UA-731, A&D Engineering).

Drugs

B9340 (molecular weight 1318.6) is a synthetic peptide antagonist of bradykinin that differs from HOE-140 (icatibant; molecular weight 1304.6) by replacement of the α-(2-indamyl)glycine at position 7 of the molecule with a tetrahydroisoquinoline-3-carboxylic acid moiety. When compared with HOE-140 in animal studies, B9340 retains similar potency of inhibition at the B1 receptor (pIC50 of 9.8 for both) but produces more than a 100-fold greater inhibition at the B2 receptor (pIC50 of 6.0 and 8.1 for HOE-140 and B9340, respectively). Pharmaceutical grade B9340 (Clinalfa AG), HOE-140 (Clinalfa), substance P (Clinalfa), and bradykinin (Clinalfa) were dissolved in 0.9% saline before infusion. All solutions were freshly prepared on the day of study.

Protocol Design

Healthy Volunteer Study

After 30 minutes equilibration with saline infusion, intra-arterial placebo (0.9% saline), B9340 at 4.5 nmol/min, or B9340 at 13.5 nmol/min was infused for 100 minutes on separate occasions in a randomized, ascending dose, double-blind manner. Placebo or B9340 was infused with saline, with bradykinin at 30, 300, and 3000 pmol/min for 10 minutes at each dose, and, after 30-minutes saline infusion, with substance P at 4, 8, and 16 pmol/min for 10 minutes at each dose. Throughout the study, FBF was measured every 10 minutes, and the final FBF measurement taken during saline infusion was defined as the baseline FBF.

Patient Study

On each of the first three occasions, B9340 was infused intra-arterially at 0.45, 1.35, 4.5, and 13.5 nmol/min for 6 minutes at each dose. Subsequently, on the final occasion in 9 patients, HOE-140 was infused intra-arterially at 0.45, 1.35, 4.5, and 13.5 nmol/min for 6 minutes at each dose. On each occasion, drug infusion was preceded by a 30-minute saline infusion, and unblinded forearm blood flow measurements were made for the last 3 minutes of each infusion period.

Data Analysis and Statistics

Mean arterial pressure (MAP) was defined as the diastolic blood pressure plus a third of the pulse pressure. Plethysmographic data were extracted from the Chart data files, and forearm blood flow was calculated for individual venous occlusion cuff inflations by use of a template spreadsheet (Excel 97, Microsoft). Recordings from the first 60 seconds after wrist cuff inflation were not used because of the variability in blood flow that this causes. Usually the last five flow recordings in each 3-minute measurement period were calculated and averaged for each arm. To reduce the variability of the blood flow data, the ratio of flows in the two arms was calculated for each time point; in effect using the noninfused arm as a contemporaneous control. The percentage change in forearm blood flow after drug administration was calculated as follows:

\[
\frac{100 \times \frac{F(i)/F(ni) - F(i)/F(ni)}{F(i)/F(ni)}}{\%}
\]
where $F(i)$ and $F(ni)$ represent measured blood flows in the infused and noninfused arms, respectively, during periods of drug ($d$) and vehicle ($v$) administration.\textsuperscript{15}

Data are presented as mean ± SEM. On the basis of the responses, dose-response shifts were calculated for the ED$_{300}$, the dose producing a 300% increase in basal forearm blood flow. Comparisons between groups were made using 2-way ANOVA and, where appropriate, the paired Student’s $t$ test. Statistical significance was taken at the 5% level.

**Results**

**Healthy Volunteer Study**

Mean age of the subjects was 25 ± 5 years, and mean body mass index was $22 ± 2$ kg/m$^2$. B9340 was well-tolerated without any adverse events. There were no significant changes in forearm blood flow in the noninfused arm, heart rate, or mean arterial pressure during the infusions (data on file). B9340 infusion alone caused no significant change in infused forearm blood flow at either dose (at 4.5 nmol/min, $8 ± 15%$; at 13.5 nmol/min, $4 ± 11%$) compared with placebo (−$7 ± 7%$: 2-way ANOVA, placebo versus high dose, $P = 0.8$).

During placebo infusion, both bradykinin and substance P caused dose-dependent vasodilatation in the infused forearm (ANOVA, $P < 0.001$ for both). At doses of 4.5 and 13.5 nmol/min, B9340 inhibited the vasodilatation produced by intra-arterial bradykinin (2-way ANOVA, $P < 0.001$ for both doses; Figure 1) and resulted in a 5-fold and 30-fold increase in the ED$_{100}$ of bradykinin respectively. B9340 infusion had no effect on substance P–induced vasodilatation (Figure 1).

**Patient Study**

Patient characteristics are shown in the Table. Two patients reported an increase in symptoms of breathlessness after withdrawal of their ACE inhibitor therapy for 1 week, which improved after recommencing therapy. During B9340 infusion, there were no significant changes in heart rate, mean arterial pressure, or baseline FBF between the two study days, although there was a trend for mean arterial pressure to be higher when withdrawn from ACE inhibitor therapy ($99 ± 4$ mm Hg versus $93 ± 4$ mm Hg, paired $t$ test, $P = 0.08$).

B9340 caused a dose-dependent vasoconstriction in the infused forearm during chronic ACE inhibition (ANOVA, $P = 0.01$; Figure 2) that was abolished after withdrawal of therapy (2-way ANOVA, ACE inhibition versus no ACE inhibition, $P < 0.001$). Moreover, B9340 again caused a dose-dependent vasoconstriction (ANOVA, $P < 0.03$) in the 9 patients who returned for an additional visit at least 4 weeks after recommencing maintenance ACE inhibitor therapy. The change in forearm blood flow was not significantly different between the first and third visits (2-way ANOVA, $P = NS$), but it was again significantly different from the response after withdrawal of ACE inhibitor therapy (2-way ANOVA, $P < 0.005$). On the final occasion, intra-arterial infusion of HOE-140 had no significant effect on basal forearm blood flow during maintenance ACE inhibitor therapy (ANOVA, $P = NS$; Figure 2).

**Discussion**

We have shown that B9340, a novel peptidic bradykinin receptor antagonist, causes a dose-dependent inhibition of bradykinin-mediated forearm vasodilatation without influencing responses to substance P or affecting basal vascular tone. In addition, we have shown for the first time that B9340 causes peripheral vasoconstriction in patients with heart failure during the maintenance of chronic ACE inhibitor therapy. We conclude that in patients with heart failure, endogenous bradykinin may contribute to the vasodilatation produced by chronic ACE inhibition.

In healthy volunteers, we have shown that B9340 selectively inhibits vasodilatation mediated by bradykinin but not substance P. Moreover, when B9340 is infused into the forearm at doses...
The discovery and subsequent development of compounds to inhibit ACE arose from research into bradykinin potentiating factor. This protein was found not only to augment the vasoconstrictor effects of Bradykinin but also to inhibit ACE. This realization came from investigation into bradykinin potentiating factor. This protein was found not only to augment the effects of bradykinin on smooth muscle but also to inhibit ACE activity. It was later realized that the enzymes responsible for bradykinin degradation (kininase II) and angiotensin II generation (ACE) are the same protein. Therefore, it is not surprising that some of the vascular effects of ACE inhibition are attributable to the action of bradykinin. Hornig et al have shown that in healthy volunteers, ACE inhibition causes an increase in the flow-associated vasodilatation of reactive hyperemia, an effect blocked by HOE-140. In sodium-depleted volunteers and patients with hypertension, Gainer et al have shown that the acute hypotensive effects of captopril are ameliorated by intravenous administration of HOE-140. In patients with heart failure maintained on ACE inhibitor therapy. We also found no significant vasocnstrictor effects of HOE-140 in patients with chronic heart failure maintained on ACE inhibitor therapy.

Des-Arg<sup>9</sup>-bradykinin, a selective B<sub>1</sub> receptor agonist, is the product of kininase I action on bradykinin. Theoretically, if ACE (kininase II) is inhibited, bradykinin will be preferentially metabolized to des-Arg<sup>9</sup>-bradykinin. Indeed, there is a suggestion that potentiation of des-Arg<sup>9</sup>-bradykinin may, in part, be responsible for some of the side-effects of ACE inhibitor therapy, including angioedema. It has been shown in dogs that infusion of des-Arg<sup>9</sup>-bradykinin causes resistance and conduit vessel dilatation. In our study, we have used a relatively nonselective bradykinin receptor antagonist, HOE-140, which, although retaining a similar activity at the B<sub>2</sub> receptor, has a 100-fold greater affinity for the B<sub>1</sub> receptor than HOE-140. Moreover, B<sub>1</sub> receptor expression on vascular endothelium is upregulated in inflammatory conditions and atherosclerosis, and there is now evidence that inflammation plays an important role in heart failure, with elevated plasma concentrations of circulating cytokines, such as tumor necrosis factor-α and interleukin-1β. Thus, the vasoconstrictor effects of HOE-140 may represent combined inhibition of the vascular actions of des-Arg<sup>9</sup>-bradykinin and ACE inhibition and may not be representative of the vascular effects during chronic ACE inhibition.

The only previous study to assess directly the contribution of bradykinin to basal vascular tone in heart failure showed an apparent, but nonsignificant, vasoconstriction with intra-arterial HOE-140 in patients treated with enalapril. The apparent disparity between our study and that by Davie et al may reflect the differing pharmacological actions of HOE-140 and B9340. To address this issue, we subsequently assessed the effects of HOE-140 in a limited number of patients, including 4 subjects who had received B9340. We also found no significant vasoconstrictor effects of HOE-140 in patients with chronic heart failure maintained on ACE inhibitor therapy.

**Figure 2.** Percentage change in forearm blood flow with intrabrachial infusion of B9340 during ACE inhibition (●, n=12), after withdrawal of ACE inhibition (●, n=12), and after reinstitution of therapy (●, n=9) in patients with heart failure. Subsequent forearm blood flow response to intrabrachial HOE-140 infusion in patients with heart failure maintained on ACE inhibitor therapy is also shown (●, n=9). There were no significant changes in forearm blood flow with B9340 in the absence of ACE inhibition or HOE-140 in the presence of ACE inhibition. *P<0.01; †P<0.03. ANOVA with repeated measures.
bradykinin at the B1 and B2 receptors. However, to date, the biological activity and functional significance of des-Arg9-bradykinin and B2 receptor–mediated effects have not been assessed in humans.

This study has wide-ranging implications for future work on ACE inhibition. If part of the benefit of ACE inhibitor therapy is attributable to increases in endogenous bradykinin and its metabolites, then even greater benefit might be obtained from combined ACE and neutral endopeptidase (NEP) inhibition. Indeed, in vivo animal and ex vivo human studies have demonstrated that the actions of bradykinin are augmented additionally by combined ACE and NEP inhibition. Moreover, the recent IMPRESS trial showed a significant benefit over lisinopril in the combined end point of death, hospital stay, or discontinuation of study medication for worsening of heart failure when patients with heart failure were treated for 24 weeks with omapatrilat, a combined ACE and NEP inhibitor. However, combined vasopeptidase inhibitors like omapatrilat also augment other peptide systems, such as the natriuretic peptides, and it remains to be established whether bradykinin provides a greater contribution to the maintenance of basal vascular tone in patients treated with these agents.

In conclusion, B9340 is a potent and specific inhibitor of bradykinin-induced peripheral vasodilation. Using B9340 at doses sufficient to increase the ED50 of bradykinin by 30-fold, we have demonstrated that bradykinin contributes to the maintenance of basal peripheral vascular tone in patients receiving chronic ACE inhibitor therapy for heart failure. Additional work is now required to determine the effects of selective B1 receptor antagonism and systemic bradykinin antagonism in this group of patients.

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