Role of Bradykinin in the Vasodilator Effects of Losartan and Enalapril in Patients With Heart Failure

Andrew P. Davie, BSc, MB, ChB, MRCP; Henry J. Dargie, MB, ChB, FRCP, FESC; John J.V. McMurray, BSc, MD, FRCP, FESC

Background—ACE inhibitors have been shown to potentiate the effects of exogenous bradykinin by inhibition of its breakdown. Despite this, there is little evidence that inhibition of endogenous bradykinin breakdown actually contributes to the effects of ACE inhibitors, or indeed, other inhibitors of the renin-angiotensin system, such as angiotensin II type I receptor (AT\textsubscript{1}) antagonists, and no evidence at all that it does so in patients with heart failure.

Methods and Results—Twelve patients with heart failure (11 male, 1 female, ages 59 to 81 years) were randomized to double-blind crossover treatment with enalapril 10 mg BID followed by losartan 25 mg BID, or the reverse, each for 5 weeks. At the end of each treatment period, forearm blood flow was measured by venous occlusion plethysmography during an intrabrachial infusion of bradykinin before and after an intrabrachial infusion of Hoe-140 (a potent, selective, and long-acting bradykinin antagonist). Bradykinin caused profound vasodilatation after enalapril (peak, 357±67%) and less after losartan (peak, 230±46%). Despite this, Hoe-140 had no discernible effects after enalapril or losartan. Similarly, this was despite the finding that Hoe-140 significantly reduced vasodilatation to bradykinin after enalapril (peak, 192±35%) and losartan (peak, 66±13%).

Conclusions—Inhibition of endogenous bradykinin breakdown does not appear to contribute to the effects of ACE inhibition or AT\textsubscript{1} antagonism in the forearm of patients with heart failure at rest, despite the very obvious effects of ACE inhibition compared with AT\textsubscript{1} antagonism on exogenous bradykinin. (Circulation. 1999;100:268-273.)

Key Words: bradykinin ■ angiotensin ■ vasodilation ■ heart failure

It has long been known that ACE is identical to kininase II, the most important pathway for degradation of bradykinin.\(^1\) Indeed, when parenterally active ACE inhibitors first became available, they were known as bradykinin-potentiating peptides.\(^2\) Parenterally active angiotensin II type I receptor (AT\textsubscript{1}) antagonists became available around the time before orally active AT\textsubscript{1} antagonists became available.\(^3\) Shortly afterward, orally active ACE inhibitors became available,\(^4\) and the focus of interest switched to their effects on the renin-angiotensin system, because it was a long time before orally active AT\textsubscript{1} antagonists became available.\(^5\) Now that orally active ACE inhibitors and AT\textsubscript{1} antagonists are both readily available, the focus of interest is switching back from their similarities to their differences. The most obvious difference remains that ACE inhibitors are bradykinin-potentiating, whereas as far as we know, AT\textsubscript{1} antagonists are not, at least not directly. Despite this, it is not known to what extent endogenous bradykinin potentiation actually contributes to the beneficial effects or side effects of ACE inhibitors in patients with heart failure. Although it is indisputable that AT\textsubscript{1} antagonists have a better side-effect profile than ACE inhibitors,\(^6\) it is not known at all whether they share the same beneficial effects overall. As work continues to develop the use of AT\textsubscript{1} antagonists in heart failure, it is important that their differences be scrutinized and the role of bradykinin better defined. Recently, a potent, selective, and long-acting bradykinin type 2 receptor (B\textsubscript{2}) antagonist, Hoe 140, has become available.\(^7,8\) Most of the clinically relevant effects of bradykinin being mediated through the B\textsubscript{2} receptor.\(^9,10\) In this study, we examined the effects of bradykinin, Hoe-140, and their combination in patients with heart failure randomized to double-blind crossover treatment with an ACE inhibitor and an AT\textsubscript{1} antagonist.

Methods

Patients and Randomization
The study was conducted with the approval of the West Ethics Committee. All patients gave written informed consent.

Twelve patients with chronic heart failure secondary to left ventricular systolic dysfunction confirmed by echocardiography were studied (left ventricular ejection fraction <40%). All patients were clinically stable on constant doses of vasodilator and diuretic drugs for ≥3 months, with no peripheral edema or pulmonary congestion, and none had uncontrolled hypertension, untreated hypercholesterolemia, or diabetes mellitus requiring insulin.

All patients were receiving treatment with a diuretic and ACE inhibitor at baseline (enalapril 10 mg BID in the majority). All medications apart from ACE inhibitor/AT\textsubscript{1} antagonist were kept constant during an intrabrachial infusion of bradykinin before and after an intrabrachial infusion of Hoe-140 (a potent, selective, and long-acting bradykinin antagonist).

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Conclusions—Inhibition of endogenous bradykinin breakdown does not appear to contribute to the effects of ACE inhibition or AT\textsubscript{1} antagonism in the forearm of patients with heart failure at rest, despite the very obvious effects of ACE inhibition compared with AT\textsubscript{1} antagonism on exogenous bradykinin. (Circulation. 1999;100:268-273.)

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Patient Characteristics

<table>
<thead>
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IHD indicates ischemic heart disease; IDC, idiopathic dilated cardiomyopathy; and LVEF, left ventricular ejection fraction.

throughout the period of study. Patient characteristics are summarized in the Table.

Patients were randomized to double-blind crossover treatment with enalapril 10 mg BID for 5 weeks, followed immediately by losartan 25 mg BID for 5 weeks, or vice versa. At the end of each 5-week study period, patients attended for venous occlusion plethysmography, having abstained from all aspirin therapy for 14 days. Patients took their usual medications except for aspirin and including enalapril/losartan 6 hours before attendance on the day of study. Patients abstained from alcohol, tobacco, and caffeine for 24 hours before each study and attended having fasted for ≥6 hours.

Bradykinin was infused at 10, 30, and 100 pmol/min for 3 minutes at each dose. Hoe-140 was infused at 7 μg/min for 30 minutes (total dose, 210 μg). This dose and rate of infusion were chosen after dose-ranging studies with both higher and lower doses to determine the minimum dose that produced ≥50% inhibition of the highest dose of bradykinin. Bradykinin was then reinfused as before. After the first study, patients immediately crossed over to the other treatment arm at 5- to 10-minute intervals throughout each study. This study protocol is summarized in Figure 1. A separate study, 4 of the 12 patients were also studied while taking their usual ACE inhibitor (enalapril 10 mg BID). Hoe-140 was infused at 89 μg/min for 15 minutes (total dose, 1335 μg). This dose and rate of infusion were shown in dose-ranging studies to produce 100% inhibition of the highest dose of bradykinin.

Measures

Studies were performed with patients lying supine in a quiet clinical laboratory maintained at a constant temperature between 23°C and 25°C. After local anesthesia with 1% lidocaine (Astra Pharmaceuticals), a 27-gauge steel needle (Terumo Medical Corp) was inserted into the nondominant brachial artery and connected to a constant-rate infusion pump (IVAC P1000, Alaris Medical Systems) via a 16-gauge epidural catheter (Portex Ltd). Physiological saline solution (0.9%, Baxter Healthcare Ltd) was infused at 1 mL/min for ≥20 minutes before drug infusion.

Blood flow was measured simultaneously in the infused and noninfused arms by venous occlusion plethysmography by use of indium/gallium-in-Silastic strain gauges applied to the widest aspect of each forearm, elevated above the level of the right atrium to assist venous outflow. To obtain blood flow measurements, hand circulation was excluded by inflation of wrist cuffs to 220 mm Hg, and upper-arm cuffs were inflated to 40 mm Hg to obstruct venous outflow for 12 of every 16 seconds. Voltage output from a Hokanson plethysmograph (Hokanson Corp) was transferred via a MacLab 4e analog-to-digital converter (AD Instruments) to a Macintosh personal computer (PowerMac, Apple Computer Inc) for analysis using Chart software (version 7.0, Microsoft Corp), averaged, and the mean percentage change from baseline in the ratio of flow between the infused and noninfused arms was calculated. Provided that blood pressure remains constant, increases in blood flow can be taken to represent vasodilatation and decreases in blood flow can be taken to represent vasoconstriction. This method is extremely well validated and uses the noninfused arm as a contemporaneous control and a means of distinguishing the effects of drug infusion from any other external or environmental factors.

Blood pressure and pulse rate were recorded manually in the noninfused arm at 5- to 10-minute intervals throughout each study.

Drugs

Bradykinin was obtained from Clinalfa AG and dissolved in normal saline. Hoe-140 was obtained from Peptides International Inc and dissolved in normal saline. Both drugs were always used within 2 hours of final preparation and destroyed thereafter.

Data Analysis

All results are expressed as mean values with 95% CIs in the text and mean values with SEMs in the figures. All results were compared by 2-tailed paired t tests. Differences were considered statistically significant at a value of P<0.05.

Results

Local infusion of bradykinin and Hoe-140 caused no adverse or systemic effects, and patients did not report any discom-
fort. Pulse rate, blood pressure, and forearm blood flow in the noninfused forearm did not change significantly during the study days, and there were no significant differences in baseline pulse rate, blood pressure, or baseline forearm blood flow between the 2 different study days (although there was a trend toward a lower blood pressure and a higher baseline forearm blood flow on enalapril study days compared with losartan study days).

**Effect of Bradykinin**

Bradykinin caused marked vasodilatation that was rapid in onset (1 minute) and almost as rapid in offset (5 minutes). There was a clear dose-response relationship, with peak vasodilatation at the highest dose of 100 pmol/min (Figure 2). There was a very obvious effect of pretreatment, with more vasodilatation to bradykinin in the enalapril limb of the study than in the losartan limb (163±33%, 248±62%, and 357±67% versus 36±10%, 103±19%, and 230±46%; P=0.0002). Indeed, vasodilatation to 100 pmol/min of bradykinin in the losartan limb of the study was not much different from that to 10 pmol/min in the enalapril limb (230±46% versus 248±62%, P=NS).

**Effect of Hoe-140**

Hoe-140 had no effect on its own in either the enalapril or the losartan limb of the study. Although there appeared to be slight vasoconstriction to Hoe-140 in the enalapril limb of the study, this was not significantly different from the response in the losartan limb, and only slightly different from baseline. Even if this were taken to signify vasoconstriction, which our study is underpowered to detect, it is extremely mild in degree (vasoconstriction at 30 minutes, 4±2%, Figure 3). Furthermore, a dose of Hoe-140 almost 7 times higher given to 4 of the 12 patients while they were taking their usual ACE inhibitor produced a similar lack of response (an apparent vasodilatation of 8±2%).

**Effect of Bradykinin After Hoe-140**

Bradykinin again caused vasodilatation, although rather less than before (Figure 2). This was not due to tachyphylaxis. At the lower doses of bradykinin, vasodilatation was almost completely abolished. Vasodilatation to bradykinin after Hoe-140 in the enalapril limb of the study was not very much different from that preceding Hoe-140 in the losartan limb (peak vasodilatation, 192±46% versus 230±46%, P=NS). Vasodilatation to bradykinin after Hoe-140 in the losartan limb of the study was very modest indeed compared with the other responses in this study (peak vasodilatation, 66±13%).

**Discussion**

In this study, we show that exogenous bradykinin causes vasodilatation in the forearm of patients with heart failure, that this response is enhanced by pretreatment with an ACE inhibitor compared with an AT1 antagonist, and that endogenous bradykinin antagonism with Hoe-140 has no significant effects on its own, irrespective of pretreatment with an ACE inhibitor or an AT1 antagonist, even though it provides effective antagonism of exogenous bradykinin. All these findings are novel, interesting in their own right, and have wider implications.

Bradykinin has been well studied in healthy volunteers and in patients with endothelial dysfunction, but not in patients with heart failure, despite the importance of ACE inhibitors and the potential importance of bradykinin potentiation in the treatment of heart failure. Our findings confirm that bradykinin is a potent vasodilator in patients with heart failure, as it is in subjects without heart failure. Furthermore, they generate the hypothesis that this may be one endotheli-
um-dependent response that is not impaired in heart failure, at least not markedly, because the responses we saw were remarkably similar to those seen previously in subjects without heart failure. This is perhaps all the more surprising given that heart failure has been reported to increase plasma levels of bradykinin. It is known that ACE inhibition increases forearm blood flow in response to bradykinin. Indeed, a previous study compared the effect of enalapril with that of losartan on the forearm blood flow response to bradykinin. Our study adds usefully to what is known about the role of bradykinin in the effects of ACE inhibitors and AT1 antagonists. Rather than healthy volunteers, our study used patients with heart failure, the very patients in whom the effects of ACE inhibitors and AT1 antagonists are of most concern. Also, we examined the effects of long-term (5 weeks) oral treatment with enalapril/losartan, rather than the short-term response examined after intra-arterial enalaprilat or a single oral dose of enalapril/losartan. It is well known that the short-term effects of ACE inhibitors may differ quite markedly from their long-term effects, which are obviously of more clinical interest. It is not known to what extent the long-term effects of AT1 antagonists might differ from their short-term effects. Despite these considerations, our findings in patients with heart failure studied after long-term treatment really are remarkably similar to those in healthy volunteers studied after short-term treatment. This is perhaps even more surprising given that ACE inhibitors have been reported to cause downregulation of bradykinin receptors. Obviously, it is possible that some sort of difference between patients and volunteers is canceled out by long-term versus short-term treatment, or vice versa. In any case, our findings confirm what has been found previously and extend those findings to a more clinically relevant setting.

It is surprising that we found no significant effect of antagonism of endogenous bradykinin with Hoe-140. It has been reported that intra-arterial Hoe-140 on its own has no significant effect on resting blood flow, although it reduces the increase in radial artery diameter (but not the increase in blood flow) after wrist arterial occlusion. The only other published report of the effects of Hoe-140 in the forearm of humans showed no hemodynamic effect of intravenous administration. These previous studies were in healthy volunteers, rather than patients with heart failure on any sort of treatment. The fact that we found no short-term hemodynamic effect of antagonism of endogenous bradykinin with Hoe-140 even in patients with heart failure, even when treated with an ACE inhibitor, suggests that there is a discrepancy between the very obvious potentiation of exogenous bradykinin by ACE inhibitors and the lack of evidence of potentiation of endogenous bradykinin. These findings are, of course, consistent with the evidence that ACE inhibitors do not increase plasma levels of bradykinin.

Our findings would initially appear to be at odds with a recent report that the short-term hemodynamic effects of oral captopril can be ameliorated by coadministration of intravenous Hoe-140. This implies that potentiation of endogenous bradykinin does contribute to the hemodynamic effects of ACE inhibition. Apart from the superficial differences between this study and our own (hypertensives versus CHF patients, captopril versus enalapril, intravenous versus intra-arterial Hoe-140), the most important difference is that of timing. Whereas Gainer et al examined the effect of Hoe-140 on the effects of a single dose of captopril, we examined the effect of Hoe-140 in patients treated with enalapril (or losartan) for 5 weeks. As we have already observed, the long-term effects of ACE inhibitors may be quite different from their short-term effects. Gainer et al made a similar observation and called for study of the role of bradykinin during long-term ACE inhibition. Our findings answer that call.
One further difference between the study by Gainer et al. and our own is that between a systemic hemodynamic study and a local hemodynamic study. Obviously, each has its strengths and weaknesses, and probably both are required for full characterization of any mechanism. The discrepancy might be construed as evidence that forearm vascular responses are not relevant to the effect of ACE inhibitors. Apart from the fact that enalaprilat has been shown to be directly vasodilating in the forearm, as have angiotensin receptor blockers, it is well established that ACE inhibitors do increase forearm blood flow in the long term if not necessarily in the short term, and even when intrabrachial enalaprilat has failed to have any effect on its own, it has increased responses to intrabrachial bradykinin.

The final part of our study confirmed that intra-arterial Hoe-140 antagonized the effects of exogenous bradykinin, despite the lack of any effect of Hoe-140 on its own. It has previously been shown that intravenous Hoe-140 antagonizes the effects of intra-arterial bradykinin and that intra-arterial Hoe-140 blocks the effect of intra-arterial quinaprilat on flow-dependent dilation in the form of reactive hyperemia. This is the first study of the interaction of intra-arterial Hoe-140 and intra-arterial bradykinin and the interaction of that interaction with long-term oral (5 weeks) treatment with ACE inhibitor/AT1 antagonist in patients with or without heart failure. The dose of Hoe-140 used was lower than in previous studies, because we deliberately sought to use the lowest dose capable of providing effective antagonism of exogenous bradykinin. A higher dose of Hoe-140 had similar effects. It is clear that the lower dose did not provide complete antagonism of exogenous bradykinin, although it did so at the lower doses of bradykinin used. It is important to note that the doses of bradykinin used were massively supraphysiological, perhaps by as much as 3 orders of magnitude, and the level of blockade achieved must therefore be regarded as similarly profound. The possibility that the reduction observed could be ascribed to tachyphylaxis can be discounted, because our own observations (unpublished data, 1998) and those of others have shown that although tachyphylaxis to bradykinin occurs, it is much more modest.

Our findings are also relevant to the ongoing controversy surrounding the potential negative interaction between ACE inhibitors and aspirin. If such an interaction exists, it is usually postulated that it is because of the effect of aspirin on prostanoid-mediated effects of bradykinin. If exogenous bradykinin potentiation turns out to be an epiphenomenon of ACE inhibition, not relevant to the effects of ACE inhibition on endogenous bradykinin activity, then there is no obvious substrate on which aspirin can exert its effects, and the proponents of this theory will have to look elsewhere for evidence to back up their hypothesis.

In summary, we have shown that exogenous bradykinin does cause vasodilatation in patients with heart failure and that this response is markedly enhanced by pretreatment with an ACE inhibitor compared with an AT1 antagonist, even though there is no evidence of an effect of endogenous bradykinin antagonism with Hoe-140, irrespective of pretreatment with an ACE inhibitor or an AT1 antagonist. Simultaneous demonstration of this discrepancy is completely novel and has never before been achieved in patients with heart failure, although it is largely consistent with previous work in vitro, in animals, and in healthy volunteers. Although this seems to confirm that AT1 antagonists should not share all of the side effects of ACE inhibitors, it leaves open the question of whether or not they will share all their beneficial effects and emphasizes how little really is known about just how both groups of compounds act.

Acknowledgments

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References


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