Bradykinin B2 Receptor Antagonism Attenuates Blood Pressure Response to Acute Angiotensin-Converting Enzyme Inhibition in Normal Men

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Abstract—The physiological effects of angiotensin-converting enzyme (ACE) inhibition may be in part mediated by bradykinin. We investigated the effect of coadministration of the specific bradykinin B2 receptor antagonist icatibant on hemodynamic and neurohormonal responses to acute intravenous ACE inhibition in normal men on a normal sodium diet. We performed a 4-phase, double-blind, double-dummy, placebo-controlled study in 12 male volunteers. The bradykinin antagonist icatibant (10 mg IV) was coadministered over the first 15 minutes of a 2-hour infusion of the ACE inhibitor perindoprilat (1.5 mg IV). Perindoprilat inhibited ACE activity and elicited the expected changes in active renin concentration and angiotensin peptides. Over the 3 hours after the start of drug infusion, perindoprilat lowered and icatibant increased mean arterial blood pressure (each $P < 0.0005$ versus placebo). Coadministration of icatibant attenuated the mean arterial blood pressure response to perindoprilat ($P < 0.0005$) but had no effect on neurohormonal responses to perindoprilat. Our study indicates that the bradykinin B2 receptor antagonist icatibant attenuates the short-term blood pressure–lowering effect of acute ACE inhibition in normal men on a normal sodium diet. Bradykinin B2 receptor antagonism alone increases resting blood pressure. Bradykinin may be involved in the control of blood pressure in the resting state in humans. (Hypertension. 2000;36:132-136.)

Key Words: blood pressure ▪ angiotensin-converting enzyme ▪ bradykinin ▪ icatibant

Angiotensin-converting enzyme (ACE) catalyzes the conversion of angiotensin I (Ang I) to angiotensin II (Ang II) and the breakdown of bradykinin (BK), a potent vasodilator.1,2 The pharmacological effects of ACE inhibitors may be in part mediated via BK accumulation rather than reduced Ang II formation.3 There are at least 2 BK receptor subtypes, B1 and B2; the known biological effect of BK is mediated via the B2 receptor,4 the stimulation of which leads to the formation of NO5 and vasodilator prostaglandins.6

Icatibant (Hoe140) is a specific B2 receptor antagonist that inhibits with high potency a variety of B2-mediated effects.7 Icatibant attenuates the hypotensive response to ACE inhibition in the dog8 and rat9,10 and inhibits BK-induced vasodilation in a dose-dependent manner in human vascular beds in vivo.11 The drug has a long duration of action: its half-life of protection against BK-induced hypotension in rats is $>$5 hours.7,10 A possible noncompetitive component to the action of the drug is suggested by flattening of the BK log–dose response curve at high doses.11,12

The purpose of the present study was to investigate the effect of coadministration of icatibant on the hemodynamic and neurohormonal responses to acute ACE inhibition in normal men. We also sought to assess the possible role of BK in basal blood pressure (BP) control in this situation. We used a protocol of constant-rate intravenous infusion of ACE inhibitor previously used by our group.13-15 By so doing, we sought to achieve rapid, long-lasting, and profound inhibition of plasma ACE. By concomitant infusion of icatibant during the first part of ACE inhibitor administration, we aimed to ensure temporal overlap of ACE inhibition and BK receptor blockade.

Methods

Study Design

We performed a randomized, 4-phase, double-blind, double-dummy, placebo-controlled study in 12 healthy male volunteers (22.9 ± 1.9 years). The study was approved by the local Ethical and Research Committee, and subjects gave written informed consent. Prestudy ECG, plasma cholesterol, and renal and hepatic function were normal in all subjects. None had previously been exposed to either study drug nor was taking any prescribed or over-the-counter medication. Subjects avoided high-salt foods, alcohol, tobacco, and caffeine for 48 hours before and during study days, which were at least 14 days apart.

Studies were performed with the subjects in a supine position. Venous cannulas were inserted for drug administration and blood sampling. Supine BP and heart rate were measured in duplicate by use of automatic sphygmomanometry (Critikon, Dynamap, Johnson & Johnson) at 2-minute intervals for 1 hour before and 3 hours after

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the start of drug infusion and thereafter at 30-minute intervals for up to 12 hours. Drugs were formulated on each day by the hospital pharmacy and were administered by constant-rate intravenous infusion (Braun Secura E, Melsungen AG). Perindoprilat (1.5 mg, Institut de Recherches Internationales Servier) or matching placebo (0.9% NaCl, Boots PLC) was infused over 120 minutes. Icatibant (10 mg, Hoechst Marion Roussel) or matching placebo (0.9% NaCl) was infused concomitantly over the first 15 minutes of ACE inhibitor infusion. In each phase, volunteers received perindoprilat + placebo, icatibant + placebo, perindoprilat + icatibant, or placebo + placebo according to a randomized-order Latin square design to avoid any systematic carryover effect.

Blood was drawn at fixed intervals for the determination of plasma ACE activity, perindoprilat concentration, active renin concentration (ARC), and Ang I and Ang II concentrations. Samples were collected into chilled tubes, placed on ice, processed immediately, and stored at −70°C until assay.

**Laboratory Analyses**

Plasma perindoprilat concentration and ACE activity were measured by high-performance liquid chromatography.16 The lower limit of detection is 0.44 nmol/L, and the interassay coefficient of variation is 8% at 12 nmol/L and 4% at 45 nmol/L. ACE activity was also estimated by use of the plasma [Ang II]/[Ang I + Ang II] ratio.15,17 Established assays for angiotensin peptide concentrations18 and ARC19 were used. Intra-assay and interassay coefficients of variation were <10%.

**Statistical Analyses**

Studies of novel pharmacological agents in normal subjects may be hampered because the absolute magnitude of the response under investigation is limited, particularly in healthy volunteer subjects. Thus, we chose to apply robust statistical analysis to the present study. For each individual in each treatment arm, we studied (1) the profile of placebo and baseline-corrected BP and heart rate (mixed model ANOVA), (2) the area under the BP/time curve (ANOVA), and (3) the mean maximal change in BP and heart rate irrespective of the time course of each (ANOVA).

Mean arterial blood pressure (MAP) was calculated from the following: MAP = diastolic BP + (systolic BP − diastolic BP)/3. We prospectively decided to analyze data from the first 3 hours after the start of drug infusion and over the full 12-hour period. A mixed-model ANOVA was fitted to all data by use of Program 3V in the BMDP package.20 The model used fixed-effect terms for treatment, start of drug infusion and over the full 12-hour period. A mixed-model ANOVA was fitted to all data by use of Program 3V in the BMDP package.20 The model used fixed-effect terms for treatment, study phase, and time within phase, assuming between-phase carryover effects to be negligible. Random-effect terms were used to model interindividual variability. Baseline BPs or heart rates, established from the mean of duplicate measurements taken at 2-minute intervals over the 20 minutes preceding the start of infusion, were included as additional fixed-effect covariates. This model allowed simultaneous estimation of both within- and between-individual factors, as well as interactions between factors. Because of extensive control for confounding factors, the model had the power to investigate minor differences between treatments.

Hemodynamic measurements after the start of study infusions were averaged within successive 10-minute periods for the first 3 hours and at fixed time points thereafter. Comparison between treatments followed baseline and placebo correction. ARC and angiotensin peptide concentrations were compared after logarithmic transformation. All values shown are mean ± 1 SD unless otherwise stated.

**Results**

All treatments were well tolerated with no adverse events. Perindoprilat concentrations were as expected from previous studies.13–15 Icatibant did not affect the peak concentration of perindoprilat (104 ± 33 nmol/L perindoprilat and 95 ± 29 nmol/L perindoprilat + icatibant, P = 0.54) or the area under the concentration/time curve for up to 12 hours (250 ± 2 nmol/L per hour perindoprilat and 250 ± 1.6 nmol/L per hour perindoprilat + icatibant, P = 0.92).

**Hemodynamic Response**

Placebo and baseline-corrected profiles of MAP with perindoprilat alone, icatibant alone, and the combination of the 2 treatments over the first 3 and 12 hours after the start of drug infusion are shown in Figures 1 and 2, respectively. As expected, there was wide interindividual variation in response to each treatment.

**Profile of MAP Response**

Over the first 3 hours, the maximum average change in MAP in response to perindoprilat alone was a fall of −3.5 ± 3.5 mm Hg at 130 minutes (Figure 1). In contrast, the maximum average change in MAP in response to icatibant alone was a rise of +2 ± 3.5 mm Hg, again seen at 130 minutes.

Application of the mixed-model ANOVA to the profile of BP response revealed differences among treatments. Over the first 3 hours, perindoprilat reduced MAP (P < 0.0005 versus...
placebo), primarily reflecting reduced diastolic BP (DBP, \( P<0.0005 \)). The fall in systolic BP (SBP) failed to reach statistical significance (\( P=0.084 \)). In contrast, infusion of icatibant alone was associated with a rise in MAP (\( P=0.001 \) versus placebo), SBP (\( P<0.0005 \)), and DBP (\( P=0.002 \)).

Coadministration of icatibant attenuated the fall in MAP to perindoprilat (\( P=0.001 \) versus perindoprilat). Similar patterns were observed for the full 12-hour period. Perindoprilat reduced and icatibant increased MAP; each effect was significant (\( P<0.0005 \) versus placebo). Over 12 hours, the attenuation by icatibant of the effect on MAP of perindoprilat alone just failed to reach significance (\( P=0.072 \)).

Over the first 3 hours, treatment with perindoprilat alone was associated with a modest increase (3 bpm, \( P<0.0005 \) versus placebo), and icatibant alone was associated with a modest reduction (\(-5 \) bpm, \( P<0.0005 \) versus placebo) in heart rate. Coadministration of icatibant did not alter the heart rate response to perindoprilat (\( P=0.059 \)). Over 12 hours, perindoprilat alone was associated with an increase in heart rate (5 bpm, \( P<0.0005 \) versus placebo) with no effect of icatibant alone (\( P=0.313 \) versus placebo) or in combination with perindoprilat (\( P=0.190 \)).

**Area Under the MAP/Time Curve**

Comparison of areas under the MAP/time curves (AUCs) to 3 hours (AUC3) indicated differences between treatments in keeping with the mixed model ANOVA (mean AUC3; perindoprilat \(-36 \) mm Hg \( \cdot \) h, icatibant \(+5.5 \) mm Hg \( \cdot \) h; and perindoprilat+icatibant \(-12.9 \) mm Hg \( \cdot \) h; \( P<0.05 \)). Analysis of the AUC to 12 hours (AUC12) revealed differences that did not achieve significance (perindoprilat \(-52 \) mm Hg \( \cdot \) h, icatibant \(-7.8 \) mm Hg \( \cdot \) h, and perindoprilat+icatibant \(-20.2 \) mm Hg \( \cdot \) h; \( P=0.058 \)).

**Mean Maximum Hemodynamic Changes**

Analysis of the mean maximum changes in MAP and heart rate has disadvantages in not correcting for time and period, as in the ANOVA model, and in being sensitive to outlying values. However, analysis of the individual mean maximum changes in BP (as opposed to the maximum group mean as in the MAP response) over 12 hours produced results similar to those in MAP and AUC.

Perindoprilat reduced MAP (9.5±3.2 mm Hg, \( P<0.005 \) versus placebo), SBP (12.8±10.3 mm Hg, \( P<0.05 \)), and DBP (9.0±3.5 mm Hg, \( P<0.005 \)). Icatibant alone increased BP (mean maximum increase in MAP 17.4±10.3 mm Hg, \( P<0.005 \); SBP 21.3±23.3 mm Hg, \( P<0.05 \); and DBP 17.3±14.1 mm Hg, \( P<0.005 \); all versus placebo). Coadministration of icatibant with perindoprilat (mean maximum reduction in MAP 9±7.2 mm Hg, SBP 18.6±16.1 mm Hg, and DBP 7.4±5.8 mm Hg) had no effect on the mean maximal response to perindoprilat alone (\( P=0.220 \)). In keeping with the results from the ANOVA model, perindoprilat alone was associated with an increase in HR (+3.8±3.5 bpm, \( P<0.0005 \) versus placebo). Icatibant alone did not affect HR (14±7.1 bpm, \( P=0.313 \) versus placebo). Coadministration of icatibant did not alter the HR response to perindoprilat (\( P=0.111 \)).

**Neurohormonal Parameters**

Baseline plasma ACE activity (perindoprilat 25.8±6.8 IU/mL, icatibant 25.5±6.7 IU/mL, perindoprilat+icatibant 26.0±6.4 IU/mL, and placebo 25.8±8.4 IU/mL; \( P=0.95 \)), baseline ARC (perindoprilat 14.8±6.6 µU/mL, icatibant 14.6±14.3 µU/mL, perindoprilat+icatibant 13.8±6.3 µU/mL, and placebo 16.1±10 µU/mL, \( P=0.91 \)), Ang I (perindoprilat 17.4±3.9 pmol/L, icatibant 16±6.6 pmol/L, perindoprilat+icatibant 17.8±5.6 pmol/L, and placebo 17.9±6.2 pmol/L; \( P=0.83 \)), and Ang II (perindoprilat 7.5±3.3 pmol/L, icatibant 8.1±9.5 pmol/L, perindoprilat+icatibant 8.3±6.3 pmol/L, and placebo 6.5±2.7 pmol/L; \( P=0.89 \)) concentrations did not differ among the study phases. Inhibition of ACE activity was profound and nearly identical with perindoprilat (mean maximum inhibition 95.4±3.7%) and perindoprilat+icatibant (mean maximum inhibition 94.7±3.3%) (Figure 3). Similarly, perindoprilat lowered the [Ang II]/[Ang I+Ang II] ratio, with the maximum change being at 2 hours and being unaffected by coadministration of icatibant (\( P=0.899 \)) (Table). Icatibant alone had no effect on plasma ACE activity (\( P=0.833 \) versus placebo) or [Ang II]/[Ang I+Ang II] ratio (\( P=0.236 \) versus placebo). Neither placebo nor icatibant had any effect on ARC (Figure 4). Coadministration of icatibant did not alter the ARC response to perindoprilat.

**Discussion**

We have demonstrated direct attenuation of the BP-lowering effects of acute ACE inhibition by the specific B2 receptor antagonist icatibant in normal men taking a normal sodium diet. Icatibant alone elicited a significant increase in MAP, consistent with the results from the ANOVA model, perindoprilat alone was associated with an increase in HR (+3.8±3.5 bpm, \( P<0.0005 \) versus placebo). Icatibant alone did not affect HR (14±7.1 bpm, \( P=0.313 \) versus placebo). Coadministration of icatibant did not alter the HR response to perindoprilat (\( P=0.111 \)).

*Table 1. Area Under the MAP/Time Curve (AUC3) and Area Under the Curve to 12 Hours (AUC12) for MAP and heart rate.\( \text{AUC3 = AUC0-3 h, AUC12 = AUC0-12 h.} \)

<table>
<thead>
<tr>
<th>Time (Hours)</th>
<th>Baseline</th>
<th>Perindoprilat</th>
<th>Icatibant</th>
<th>Perindoprilat+Icatibant</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 h</td>
<td>117±21%</td>
<td>7.7±5.2%*</td>
<td>118±16%</td>
<td>16±14%*</td>
</tr>
</tbody>
</table>

Activity at 2 hours is expressed as percentage of baseline activity.

*\( P<0.005 \) vs placebo.

**Figure 3.** Baseline-corrected inhibition of ACE activity in the first 12 hours after 1 mg IV perindoprilat (▲), 10 mg IV icatibant (●), placebo (●), or 1 mg IV perindoprilat+10 mg IV icatibant (□) in healthy male volunteers. \( P<0.05 \) vs placebo.
to ACE inhibition. Our results provide the first evidence of a role for BK in BP homeostasis in normal men and in the response to acute ACE inhibition.

The extent of contribution of BK accumulation to the hemodynamic effects of ACE inhibition has long been debated. In models of renovascular hypertension, B2 receptor antagonism attenuates the BP-lowering effects of ACE inhibition but, in contrast, has no effect on the hemodynamic response to ACE inhibition in kinin-deficient or genetically hypertensive animals. Such studies have yielded limited support for the theory that endogenous BK contributes to BP control in the resting state in renovascular hypertension and in normotensive rats. In the rat, ACE inhibition at doses without effect on BP prevents and reverses left ventricular hypertrophy.

In humans, BK contributes to ACE inhibitor–induced vasodilation in radial and coronary arteries. The attenuation of vascular endothelial dysfunction by ACE inhibition appears to result from BK potentiation. Local BK production may be involved in the control of BP in hypertensive subjects. Attenuation by aspirin and indomethacin of the vasodilator effects of ACE inhibition in heart failure suggests the involvement of BK. However, a recent study suggested that endogenous BK makes no contribution to the vasodilator effect of chronic ACE inhibition in heart failure.

In a recent single-blind study, icatibant attenuated the acute hemodynamic effect of oral captopril by 50% in normotensive and hypertensive subjects on a low-salt diet. Our results are broadly in keeping with these, but there are a number of important differences. In the previous study, icatibant abolished the rise in plasma renin activity with captopril. We found no evidence that the renin response to ACE inhibition is dependent on BK. In salt-depleted subjects, acute ACE inhibition elicits a small but reproducible fall in BP. The similar magnitude of BP fall with oral captopril seen in normal volunteers and in hypertensive subjects in the study of Gainer et al is surprising. We deliberately avoided using a protocol of either salt repletion or depletion because of the possible confounding effects on the activity of the renin-angiotensin system. This may explain the lesser attenuation of the effect of the ACE inhibitor in normal subjects in the present study, ~20%, compared with 50% attenuation seen in salt-depleted hypertensive subjects. The results of our robust ANOVA model and the less sophisticated analysis of the AUC are consistent in showing a rise in BP with icatibant alone and attenuation with this agent of the BP-lowering effects of ACE inhibition. The vasodilator effect of BK in human resistance vessels in vivo is at least partly mediated by NO. Icatibant blocks ACE inhibitor–stimulated production of NO in isolated porcine coronary vessels. Our results are compatible with the potentiation of BK and increased NO production after ACE inhibition.

The present study may be criticized on the basis of our failure to address a possible nonspecific vasoconstrictor effect of icatibant. To our knowledge, icatibant has not previously been administered to normal subjects in the doses used in the present study. A previous study in human subjects showed no effect of up to 100 μg/kg icatibant on BP or heart rate. Noncompetitive antagonism of the B2 receptor by icatibant occurs in vitro and a similar effect in vivo is suggested by flattening of the dose-response curve in human subjects. Gainer et al infused icatibant with the ACE inhibitor but did not administer icatibant alone. These authors also studied the effects on BP of losartan (an angiotensin receptor antagonist) administered orally but did not study the combination of losartan with icatibant. Thus, these authors failed to address the possibility of either a specific or nonspecific vasoconstrictor effect of icatibant. Our findings of an increase in BP and reduction in heart rate with icatibant are equally well explained by specific BK receptor blockade. Our finding of an increase in heart rate after acute ACE inhibition is at odds with findings from previous studies in our unit in patients with heart failure and in salt-depleted volunteers. There is no clear explanation for this observation.

It has been suggested that the B2 receptor has inherent activity in the absence of agonist and that icatibant may act as an inverse agonist, stabilizing and inactivating the receptor. To the best of our knowledge, inverse agonism has been demonstrated for icatibant in cultured rat myometrial cells but never in human tissue in vitro or in vivo. Moreover, such an effect would not explain the differing effects on BP of icatibant alone compared with icatibant given with ACE inhibitor. Finally, it may be suggested that acute dosing studies do not reflect chronic ACE inhibitor use in clinical practice. This (and other methodological differences) may explain similarities and differences between our study and others.

In summary, we have demonstrated attenuation by icatibant, the specific BK B2 receptor antagonist, of the BP-lowering effect of acute ACE inhibition in normal men. Our results suggest a role for endogenous BK in BP homeostasis in normal men. The physiological relevance of these findings, in particular with respect to the hemodynamic and therapeutic responses to ACE inhibitors in clinical practice, in terms of hemodynamic response and morbidity/mortality, is unclear. Further studies in patients in which the renin-angiotensin system is activated, in particular in the setting of heart failure and in the setting of chronic ACE inhibition, may help to
clarify the role of BK in the pathophysiological effects of ACE inhibition.

Acknowledgments

This study was supported by a grant from Institut de Recherches Internationales Servier (IRIS), Courbevoie, France. Perindoprilat was a gift from IRIS. Hoe 140 was a gift from Hoechst Marion Roussel, Frankfurt, Germany.

References


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doi: 10.1161/01.HYP.36.1.132

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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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