Bradykinin Receptor Antagonism and Endothelial Tissue Plasminogen Activator Release in Humans

Fraser N. Witherow, Pamela Dawson, Christopher A. Ludlam, David J. Webb, Keith A.A. Fox, David E. Newby

Objective—We sought to assess pharmacodynamic responses to the bradykinin antagonist B9340 and to determine the contribution of the endothelial bradykinin receptor to stimulated tissue plasminogen activator (t-PA) release in humans.

Methods and Results—Bilateral forearm blood flow and plasma fibrinolytic variables were measured in 8 volunteers during 100 minutes of intrabrachial infusions of saline placebo, B9340 at 4.5 nmol/min, or B9340 at 13.5 nmol/min. On each occasion, intra-arterial bradykinin (30 to 3000 pmol/min) and substance P (4 to 16 pmol/min) were coinfused for 10 minutes at each dose. To assess the onset and offset of action, 6 additional subjects on 2 occasions received intra-arterial bradykinin (100 pmol/min) for 60 minutes with a coinfusion of either saline placebo or B9340 (13.5 nmol/min) for 12 minutes. During placebo infusion, bradykinin and substance P caused dose-dependent vasodilatation in the infused forearm ($P<0.001$). B9340 caused a dose-dependent inhibition of bradykinin-induced forearm vasodilatation and t-PA release ($P<0.001$) without affecting substance P–induced vasodilatation or t-PA release ($P=NS$). B9340 caused a reversible inhibition of bradykinin-induced vasodilatation ($P<0.001$) with a rapid onset and offset of action.

Conclusions—B9340 is a potent, reversible, and selective competitive receptor antagonist of bradykinin-induced vasodilatation and t-PA release in humans. (Arterioscler Thromb Vasc Biol. 2003;23:1667-1670.)

Key Words: bradykinin ■ blood flow ■ tissue plasminogen activator ■ receptor antagonism

Bradykinin is an endogenous, vasoactive, nonapeptide mediator involved in many physiologic processes. It is cleaved from high-molecular-weight kininogen during the contact phase of blood coagulation, resulting in endothelium-dependent vasodilatation and stimulation of tissue plasminogen activator (t-PA) release from human endothelial cells. It has a brief duration of action (plasma half-life of 15 to 30 seconds) owing to its rapid degradation by several enzymes, principally angiotensin-converting enzyme (ACE). Bradykinin appears to contribute to the vascular effects of ACE inhibitor therapy in hypertension and heart failure.

Bradykinin receptor antagonists have been developed from peptide analogues of bradykinin. The most widely used bradykinin receptor antagonist is HOE-140, or icatibant, which demonstrates high selectivity for the B$_2$ receptor. Recently, a third-generation synthetic peptide antagonist of bradykinin, B9340, has been synthesized. It has a similar chemical structure to HOE-140 and differs by replacement of the $\alpha$-(2-indanyl)glycine at position 7 of the molecule with a tetrahydroisoquinoline-3-carboxylic acid moiety. In comparison with HOE-140, B9340 retains similar potency of inhibition at the bradykinin B$_2$ receptor (median inhibitory concentration [IC$_{50}$] of 0.158 nmol/L for both) but has greater inhibition at the B$_1$ receptor (IC$_{50}$ of 1000 nmol/L and 7.9 nmol/L for HOE-140 and B9340, respectively).

The purpose of this study was to assess pharmacodynamic responses to the novel bradykinin antagonist B9340 and to determine the contribution of the endothelial bradykinin receptor to stimulated t-PA release in vivo in humans. To assess the selectivity of B9340, we compared the vasomotor and fibrinolytic responses of bradykinin with those of substance P, a bradykinin receptor–independent, endothelium-dependent vasodilator and stimulator of t-PA release.

Methods

Study Subjects

Fourteen healthy, male volunteers were recruited into the study, which was performed with approval of the local ethics committee, in accordance with the Declaration of Helsinki and the written, informed consent of each subject. Each volunteer was studied at the same time of day and had been fasting for at least 4 hours before each study. All subjects avoided alcohol for 24 hours and caffeine-containing products for 5 hours before study. No medications or vasoactive drugs were taken in the 7 days before each study.
Intra-Arterial Administration
All studies were performed in a quiet, temperature-controlled (22°C to 25°C) room. On each occasion, the brachial artery of the nondominant arm was cannulated with a 27-gauge needle (Cooper’s Needle Works Ltd) after 1% lidocaine local anesthesia. The needle was attached to a 16-gauge epidural catheter (Portex Ltd), and needle patency was maintained by an infusion of 0.9% saline at 1 mL/min. The total rate of intra-arterial infusions was maintained constant throughout all studies at 1 mL/min.

FBF and Blood Pressure
Forearm blood flow (FBF) was measured by mercury-in-silicone elastomer strain-gauge venous-occlusion plethysmography, as previously described.2,4 Immediately after each FBF measurement, pulse and blood pressure were measured noninvasively in the noninfused arm throughout each study with a semiautomated digital sphygmomanometer (UA-731, A&D Engineering). Mean arterial pressure was defined as the diastolic blood pressure plus one third of the pulse pressure.

Drugs
Pharmaceutical-grade B9340 (Clinalfa AG), substance P (Clinalfa), and bradykinin (Clinalfa) were dissolved in 0.9% saline before infusion. All solutions were freshly prepared on the day of study.

Venous Sampling and Assays
Venous cannulas (17 gauge) were inserted into large subcutaneous veins of the antecubital fossa in both arms, as described previously.9 Ten milliliters of blood was withdrawn simultaneously from each arm and collected into acidified, buffered citrate (Biopool Stabilyte) tubes and kept on ice before being centrifuged at 2000g for 30 minutes at 4°C. Platelet-free plasma was decanted and stored at −80°C before assay. Plasma t-PA antigen and activity concentrations were determined by ELISA (Coaliza t-PA, Chromogenix AB) and a photometric method (Coatest t-PA, Chromogenix AB), respectively, as described previously.10

Study Design
Protocol 1: Efficacy and Selectivity of B9340
Eight healthy, male volunteers attended on 3 occasions at least 1 week apart. 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, double-blind, ascending-dose design. Placebo/B9340 was infused with saline for 10 minutes; bradykinin at 30, 300, and 3000 pmol/min for 10 minutes at each dose; and after a 30-minute saline infusion, substance P at 4, 8, and 16 pmol/min for 10 minutes at each dose. FBF and mean arterial pressure were measured every 10 minutes for the duration of the study. Venous samples were obtained at baseline, after 10 minutes of B9340/ placebo infusion, and after each dose of bradykinin and substance P.

Protocol 2: Onset and Offset of B9340 Action
Six additional healthy, male volunteers attended on 2 occasions at least 1 week apart. After 30 minutes’ equilibration with saline infusion, intrabrachial bradykinin was infused at 100 pmol/min for 60 minutes. After 12 minutes of bradykinin infusion, B9340 (Clinalfa) at 13.5 nmol/min or placebo (saline vehicle) was infused for 12 minutes in a randomized, double-blind manner. FBF and mean arterial pressure were measured every 6 minutes for the duration of the study.

Data Analysis and Statistics
Plithysmographic data were extracted from the software (Chart) data files, and FBF was calculated for individual venous-occlusion cuff inflations by use of a template spreadsheet (Excel 97, Microsoft). The last 5 flow recordings in each 3-minute measurement period were calculated and averaged for each arm. The effective dose causing a 100% increase in FBF (ED100) was calculated to assess the degree of bradykinin antagonism. The percentage increase in blood flow was determined from the ratio of the FBFs, as described previously.8

Estimated net release of t-PA antigen and activity was defined as the product of the infused forearm plasma flow (based on the hematocrit [Hct] and the infused FBF) and the concentration difference between the infused ([t-PA]Inf) and noninfused forearms ([t-PA]Noninf): estimated net t-PA release=−BF ×(1−Hct)× ([t-PA]Inf−[t-PA]Noninf).

Results
The infusions were well tolerated with no major adverse events. There were no significant changes in FBF in the noninfused arm, heart rate, or blood pressure during the infusions (data not shown).

Protocol 1: Efficacy and Selectivity of B9340
Subjects were aged 25±5 years with a body mass index of 22±2 kg/m².

Forearm Blood Flow
Baseline blood flow during saline infusion was 2.0±0.2 mL · 100 mL⁻¹ · min⁻¹ in the infused arm and 2.0±0.1 mL · 100 mL⁻¹ · min⁻¹ in the noninfused arm. In comparison with placebo, B9340 infusion alone caused no significant change in the infused FBF from baseline at either dose (placebo, −7.4±3.7%; B9340 at 4.5 nmol/min, 8.1±7.6%; and B9340 at 13.5 nmol/L, 3.8±5.5%; ANOVA, P=0.8). During placebo infusion, bradykinin and substance P caused dose-dependent vasodilatation in the infused forearm (ANOVA, P<0.001 for both; Figure 1). At doses of 4.5 and 13.5 nmol/min, B9340 caused 7-fold and 18-fold increases in the ED100 for bradykinin, respectively (ANOVA, P<0.001 for both; Figure 1). In contrast, B9340 had no effect on substance P–induced vasodilatation (Figure 1).

Estimated Net t-PA Release
Baseline plasma t-PA antigen and activity concentrations during saline infusion were 3.4±0.4 ng/mL and 1.3±0.3 IU/mL in the infused arm and 3.6±0.3 ng/mL and 1.3±0.3 IU/mL in the noninfused arm, respectively. B9340 infusion...
alone produced no change in plasma t-PA antigen or activity (ANOVA, \( P=0.9 \)). Both bradykinin and substance P produced a dose-dependent increase in plasma t-PA antigen and activity concentrations in the infused forearm (ANOVA, \( P<0.001 \)). Both doses of B9340 completely inhibited t-PA antigen and activity release at 300 pmol/min bradykinin and reduced t-PA antigen and activity release at 3000 pmol/min by 4- to 8-fold (ANOVA, \( P<0.001 \) for both doses; Figure 2). There was no effect of B9340 on substance P-induced t-PA release (Figure 2).

**Protocol 2: Onset and Offset of B9340 Action**

Subjects were aged 30\(\pm2\) years with a body mass index of 21\(\pm2\) kg/m\(^2\). Baseline blood flow during saline infusion was 3.4\(\pm1.0\) mL \(\cdot\) 100 mL\(^{-1}\) \(\cdot\) min\(^{-1}\) in the infused arm and 2.7\(\pm0.6\) mL \(\cdot\) 100 mL\(^{-1}\) \(\cdot\) min\(^{-1}\) in the noninfused arm. Bradykinin infusion caused a 288\(\pm7\)% increase in the infused FBF that was sustained for 60 minutes (ANOVA, \( P<0.001 \); Figure 3). Compared with placebo, B9340 coinfusion caused a rapid onset and offset of inhibition of bradykinin-induced vasodilatation (ANOVA, \( P<0.001 \); Figure 3).

**Discussion**

We have confirmed our earlier findings\(^{11,12}\) that intrabrachial bradykinin infusion causes marked forearm vasodilatation and endothelial t-PA release. We have demonstrated that B9340 is a potent and competitive antagonist of bradykinin-induced vasodilatation and endothelial t-PA release in vivo in humans. B9340 appears to be a selective and reversible bradykinin receptor antagonist with a rapid onset and offset of action.

B9340 caused dose-dependent inhibition of bradykinin-induced vasodilatation and t-PA release, suggesting that it acts as a competitive receptor antagonist and that both vascular effects are mediated through bradykinin receptors. Previous clinical studies\(^{11,12}\) have used systemic intravenous administration of the selective B\(_2\) receptor antagonist HOE-140, combined with intrabrachial bradykinin infusions. However, systemic drug administration might have ancillary effects, and the antagonist effects of HOE-140 could have been mediated through intermediary pathways. In contrast, here we have demonstrated a direct, local, dose-dependent inhibition of the vascular actions of bradykinin with intrarterial infusions of B9340. Moreover, those previous studies of systemic HOE-140 administration either lacked a control vasodilator\(^{14}\) or used the endothelium-independent vasodilator sodium nitroprusside.\(^{13}\) We have more rigorously demonstrated the selectivity of bradykinin antagonism by B9340 through comparison with the tachykinin substance P, which acts through the endothelial neurokinin type 1 receptor to cause endothelium-dependent vasodilatation\(^6\) and t-PA release. The antagonist action of B9340 cannot, therefore, be attributed to a nonspecific effect on the vascular endothelium and appears to be selective for bradykinin receptors.

Bradykinin is thought to exert its vasodilatory effects by activating the B\(_2\) receptor on vascular endothelium. This results in release of nitric oxide\(^{15,16}\) and endothelium-derived hyperpolarizing factor to produce vasorelaxation.\(^{17,18}\) However, although inhibition of nitric oxide synthase with N\(^\text{G}\)-monomethyl-L-arginine attenuates the vasodilatation to bradykinin, it does not affect endothelial t-PA release.\(^{13}\) This would suggest that bradykinin-stimulated t-PA release is mediated through a nitric oxide synthase-independent pathway and that the endothelium regulates blood flow and t-PA release through distinct pathways. Indeed, we have recently demonstrated that direct, local, endothelial t-PA release can be induced by tumor necrosis factor-\(\alpha\) in the absence of alterations in blood flow in the human forearm.\(^{19}\) Thus, bradykinin-induced t-PA release appears to be dependent on the endothelial bradykinin receptors and to act through a second-messenger pathway that is distinct from the regulation of vasomotion.

B9340 had no effect on basal FBF or plasma t-PA concentrations, suggesting that in healthy humans, bradykinin does not contribute to the basal maintenance of vascular tone or t-PA release. However, this does not preclude a potential role for bradykinin in pathophysiologic conditions, such as inflammation,\(^{20}\) or instances where ACE inhibitor therapy is used.\(^{4,21}\)

Although B9340 antagonizes the B\(_2\) receptor at concentrations nearly 2 orders of magnitude greater than those at the B\(_1\) receptor, B9340 might still produce some blockade of the B\(_1\) receptor. The B\(_1\) receptor was previously thought to be inactive in the vascular system unless upregulated during

**Figure 3.** Infused-arm FBF with bradykinin infusion (black bar; 100 pmol/min) during placebo (open circles) or B9340 (closed circles; 13.5 nmol/min) coadministration (gray bar).
inflammation. Raidoo et al have recently shown that B receptors are present in large numbers on the vascular endothelium and are further upregulated around atherosclerotic plaques. However, the functional significance of the B receptor on the endothelium is unknown, and the work by Brown et al using HOE-140 would suggest that the observed effects are mediated principally through blockade of the B receptor.

Conclusions
B9340 is a potent, reversible, and selective competitive inhibitor of bradykinin-induced vasodilatation and t-PA release in humans. This compound is a potentially useful investigational tool in dissecting out the physiologic and pathophysiologic role of bradykinin in vivo in humans.

Acknowledgments
Dr Fraser Witherow (FS/2000005) and this work (PG/97197) were supported by a Research Leave Fellowship from the Wellcome Trust funded by the British Heart Foundation. Prof David Webb was supported by a Research Leave Fellowship from the Wellcome Trust (WT 0526330).

References

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Arterioscler Thromb Vasc Biol. 2003;23:1667-1670; originally published online July 17, 2003; doi: 10.1161/01.ATV.0000087142.99472.F6
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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