Bradykinin Receptor Antagonism and Endothelial Tissue Plasminogen Activator Release in Humans

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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
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) and citrate (Monovette, Sarstedt) 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
Plethysmographic 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.

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 forearm ([t-PA]Noninf): estimated net t-PA release = FBF · (1 − Hct) · ([t-PA]Inf − [t-PA]Noninf). Data were examined, where appropriate, by ANOVA with repeated measures and 2-tailed paired Student’s test with commercially available software (Excel 97). All results are expressed as mean ± SEM. Statistical significance was taken at the 5% level.

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±2 years with a body mass index of 21±2 kg/m². Baseline blood flow during saline infusion was 3.4±1.0 mL · 100 mL⁻¹ · min⁻¹ in the infused arm and 2.7±0.6 mL · 100 mL⁻¹ · min⁻¹ in the noninfused arm. Bradykinin infusion caused a 288±7% 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 findings11,12 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 studies11,12 have used systemic intravenous administration of the selective B₂ 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 intra-arterial infusions of B9340. Moreover, those previous studies of systemic HOE-140 administration either lacked a control vasodilator14 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 vasodilatation14 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 B₂ receptor on vascular endothelium. This results in release of nitric oxide15,16 and endothelium-derived hyperpolarizing factor to produce vasorelaxation.17,18 However, although inhibition of nitric oxide synthase with Nω-nitro-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-α 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 B₂ receptor at concentrations nearly 2 orders of magnitude greater than those at the B₁ receptor, B9340 might still produce some blockade of the B₁ receptor. The B₁ receptor was previously thought to be inactive in the vascular system unless upregulated during
inflammation. Raidoo et al \cite{20} have recently shown that B2 receptors are present in large numbers on the vascular endothelium and are further upregulated around atherosclerotic plaques. However, the functional significance of the B2 receptor on the endothelium is unknown, and the work by Brown et al \cite{13} using HOE-140 would suggest that the observed effects are mediated principally through blockade of the B2 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.

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References

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