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Inhibition of bradykinin-induced vasodilation in human forearm vasculature by icatibant, a potent B_2-receptor antagonist

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1 The effect of icatibant (D-Arg-[Hyp^3, Thi^5, D-Tic^7, Oic^8] bradykinin) a potent B_2-kinin receptor antagonist, was studied on bradykinin-induced vasodilation in the human forearm.

2 Eight healthy normotensive men were studied in a rising dose random-placebo controlled study. Placebo and icatibant (20, 50 and 100 μg kg^{-1} i.v.) were administered double-blind. Forearm blood flow was measured by venous occlusion plethysmography during rising dose brachial artery infusions of bradykinin (10–3,000 ng min^{-1}) 60–90 min after placebo or icatibant.

3 Plasma concentrations of icatibant fell exponentially following each of three doses, up to the final measurement. Elimination half-lives calculated from linear regression of the mean data were 25, 27 and 29 min after 20, 50 and 100 μg kg^{-1} doses respectively.

4 Icatibant inhibited the effect of bradykinin ($P < 0.001$ at each dose of icatibant) in a dose-dependent manner. Bradykinin (100 ng min^{-1}) increased mean blood flow in the infused arm by 238 ± 31% when infused following placebo, by 112 ± 21% after icatibant 20 μg kg^{-1}, by 71 ± 14% after icatibant 50 μg kg^{-1} and by 48 ± 9% after icatibant 100 μg kg^{-1}.

5 These results demonstrate that icatibant antagonises B_2-receptor mediated vasodilation in human forearm resistance vessels. The findings provide a quantitative basis for future studies of the role of bradykinin in the response to angiotensin converting enzyme inhibitors and in circulatory disease.

Keywords icatibant bradykinin B_2-receptors forearm blood flow man

Introduction

Bradykinin is a nonapeptide with diverse biological actions that exhibit marked regional and species differences [1–3]. It causes vasodilation in human resistance vessels [4] and veins [5]. Bradykinin stimulates biosynthesis of vasodilator prostaglandins in human subjects [6, 7], but pharmacologic inhibition of prostaglandin biosynthesis has little effect on its haemodynamic effects in man [8–10]. In some species it releases the endothelium derived relaxing factor (EDRF) [11] now known to be nitric oxide (NO) [12, 13] or closely related to it, and some at least of its vasodilator effect in man is probably mediated by EDRF/NO [14]. There is evidence that local production of bradykinin is involved in the control of blood pressure in human and experimental hypertension [15, 16], and evidence that some of the actions of angiotensin converting enzyme (ACE) inhibitors are due to enhanced endothelial autacoid formation as a result of inhibition of breakdown of endothelium-derived bradykinin [17].

At least two distinct bradykinin receptors (B_1 and B_2) have been identified. There are marked tissue differences and differences between health and disease, as well as species differences, in regard to the relative importance of these receptors [2, 18]. Much of the biological activity of bradykinin in healthy human

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tissues appears to be mediated via B_2-receptors, but investigation of the roles of bradykinin in health and disease have been hampered until recently by a paucity of potent B_2-antagonists. The first sequence related competitive B_2-receptor antagonist was described by Vavrek & Stewart [19] and many analogues based on their work have been synthesised subsequently in efforts to improve potency. A breakthrough was achieved by Henke and colleagues in 1990 [20] who introduced several unnatural amino acids that had been used during the development of potent ACE inhibitors. The compound that they synthesised, D-Arg-[Hyp^3, Thi^5, D-Tic^7, Oic^8] bradykinin (approved name icatibant, previously known as HOE 140), exceeds the potency of earlier antagonists by 2–3 orders of magnitude and represents a new class of B_2-receptor antagonist. *In vitro* studies have shown a pA_2 value in guinea-pig ileum of 8.42, and receptor binding studies have confirmed the presence of high affinity binding sites for icatibant in this tissue [21]. Icatibant also inhibits a variety of other B_2 mediated responses, including those of rat uterus, guinea-pig pulmonary artery, and release of EDRF and prostacyclin from cultured bovine aortic endothelial cells with comparably high potency [21]. This antagonist has undergone animal toxicity testing to a point that has enabled it to be administered to man, and has been well-tolerated in phase I pharmacokinetic studies (J. Collins, personal communication, Hoechst UK). The objectives of the present study were to assess the quantitative and qualitative effects of icatibant on intra-arterial bradykinin induced vasodilatation on forearm blood flow in healthy male volunteers. A secondary objective was to relate these quantitative data to plasma icatibant concentrations.

Methods

Subjects and protocol

Experiments were performed on eight healthy men (aged 18–34 years, body mass index 19.4–25.4 kg m^{-2}) who gave their written informed consent to take part in the study, which had the approval of Lewisham and North Southwark Ethics Committee. Before entry all were screened with medical history, physical examination, electrocardiogram, haematological and biochemical screen, urinalysis, drug and alcohol screen and hepatitis serology. No clinically significant abnormalities were detected. Subjects were studied on four separate occasions at least 1 week apart, and on each occassion were admitted to the Guy's Drug Research Unit for two nights (the night before and the night after each study day).

An intravenous cannula was inserted, and subjects infused intravenously over 60 min with icatibant (20, 50 or 100 μg kg^{-1} body weight) or placebo (consisting of physiological saline, the vehicle for icatibant) according to a rising dose, randomised, double-blind protocol. Icatibant (molecular weight 1304.59) was obtained from Hoechst, UK as a concentrated solution (1 mg ml^{-1}) and stored at 4° C. It was diluted in physiological saline (NaCl 140 mmol l^{-1}) immediately before infusion. Immediately after the end of the intravenous infusion period subjects were transferred to a quiet clinical laboratory and rested supine for a minimum of 30 min. Room temperature (21–24° C) was maintained constant ± 1° C, for each study. A 27 gauge steel cannula (Coopers Needle Works, Birmingham, UK) was inserted into the left brachial artery under local anaesthesia using less than 1 ml of 1% lignocaine hydrochloride (Antigen Limited, Ireland). Forearm blood flow was measured in both arms using venous occlusion plethysmography with temperature-compensated mercury-in-silastic strain gauges [22] as described previously [10] and interfaced with a personal computer. Saline (NaCl 140 mM, Travenol, Thetford UK), icatibant and bradykinin were infused at a constant rate of 1.0 ml min^{-1}. Saline was administered 102 min after the start of the icatibant/placebo infusion, via the arterial needle for two baseline periods (each of 6 min) followed by rising doses of bradykinin, each for 6 min (10, 30, 100, 300, on all occasions and 1,000 and 3,000 ng min^{-1} unless an estimated doubling of forearm blood flow over that expected in the absence of antagonist had occurred). The recording period for the lowest dose of bradykinin was concluded 60 min after the infusion of icatibant was discontinued, and the recording period of the highest dose of bradykinin (if the 3,000 ng min^{-1} dose was reached) concluded 90 min after the finish of the icatibant/placebo infusion. Flows were recorded for 10 s in every 15 s during the final 3 min of each infusion period and the mean of the final five measurements used for analysis. Blood pressure was measured manually using a mercury sphygmomanometer, and heart rate determined from the radial pulse immediately before the start of the placebo/icatibant infusion and then at 30 min intervals until completion of the bradykinin infusion.

Plasma icatibant assay

Venous blood (5 ml in lithium heparin tubes) was sampled via an intravenous cannula in the right arm for determination of plasma icatibant concentrations. Blood samples were drawn immediately before and after the infusion of placebo/icatibant and then at 30 min intervals until completion of the bradykinin infusion. Samples were assayed by Hoechst using radioimmunoassay.

Statistical analysis

Results are presented as means (± s.e. mean). Data were analysed by analysis of variance (ANOVA) for repeated measures. Differences were considered significant when $P < 0.05$.

Results

No subject had any clinically important adverse experiences during the study day and up to discharge

the following day. The following events were reported: subject 1 reported upper respiratory symptoms on three study days (placebo, icatibant 20 and icatibant 100 μg kg^{-1} days) and drowsiness and lightheadedness lasting 3.5 h on the icatibant 50 μg kg^{-1} day; subject 2 reported pain in his left forearm lasting 1 min on the icatibant 50 μg kg^{-1} day and back pain lasting 25 h on the placebo day; subject 5 was noted to have an inflamed cannula site on the icatibant 100 μg kg^{-1} day; and subject 8 had a headache lasting 14 h on the icatibant 20 μg kg^{-1} day. All adverse experiences were graded as 'mild' (rather than 'moderate' or 'severe'). No clinically significant abnormalities in urinalysis, haematology or biochemistry determinations occurred. There was no significant change in mean blood pressure (range 82.9–91.1 mm Hg) or heart rate on any study day.

Plasma concentrations of icatibant fell exponentially following each of the three doses, up to the final measurement (Figure 1). Elimination half-lives calculated from linear regression of the mean data were 25, 27 and 29 min after 20, 50 and 100 μg kg^{-1} doses respectively.

Blood flow was stable during the baseline periods (6–12 min). Intra-arterial bradykinin increased forearm blood flow in the infused arm without significant change in the non-infused arm on any of the four study days. On the placebo day blood flow in the infused arm increased from a baseline of 3.1 ± 0.3 during saline to 16.8 ± 2.2 ml 100 ml^{-1} min^{-1} during bradykinin (300 ng min^{-1}). Icatibant inhibited the effect of bradykinin ($P < 0.001$ at each dose of icatibant) in a dose-dependent manner. Bradykinin (100 ng min^{-1}) increased mean blood flow in the infused arm by 238 ± 31% when infused following placebo, by 112 ± 21% after icatibant 20 μg kg^{-1}, by 71 ± 14% after icatibant 50 μg kg^{-1} and by 48 ± 9% after icatibant 100 μg kg^{-1}. Figure 2 shows semi-logarithmic plots of mean ± s.e. mean forearm blood flow *vs* bradykinin dose on each of the three icatibant days compared with the dose-response on the placebo day.

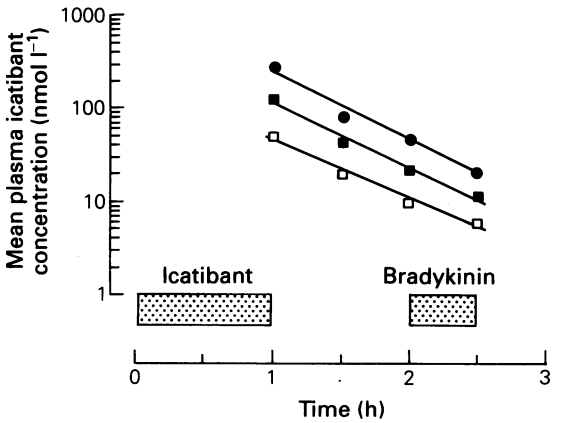

Figure 1 Mean plasma icatibant concentrations (nmol l^{-1}, $n = 8$) following a 1 h intravenous infusion of icatibant (indicated by shaded bar) at doses of 20 μg kg^{-1} (open squares), 50 μg kg^{-1} (closed squares) and 100 μg kg^{-1} (closed circles). Pharmacodynamic measurements were made as indicated between 2–2.5 h .

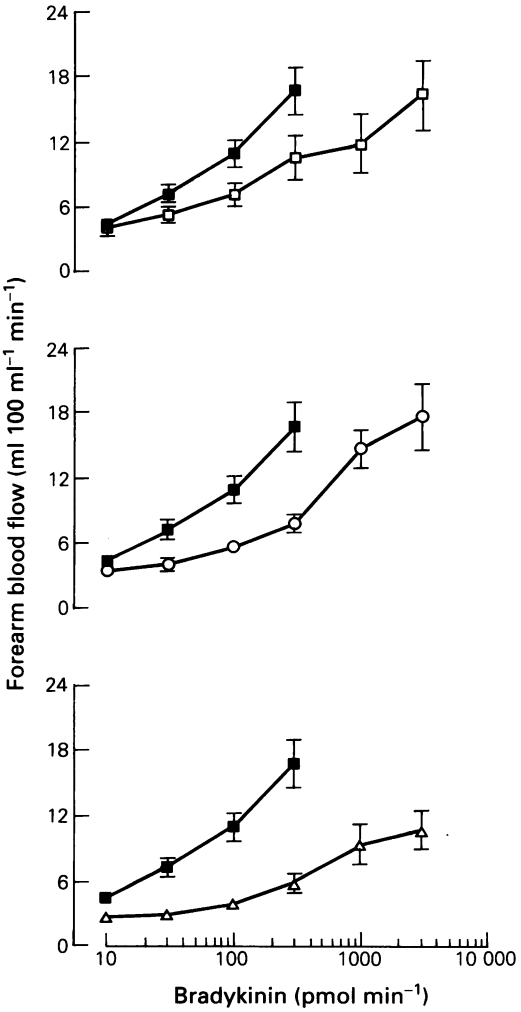

Figure 2 Forearm blood flow responses to intra-arterial infusion of bradykinin following intravenous infusion of placebo (closed squares) or icatibant 20 μg kg^{-1} (open squares), 50 μg kg^{-1} (open circles) and 100 μg kg^{-1} (open triangles). Data are mean ± s.e. mean ($n = 8$).

There was a shift to the right of the bradykinin log dose-response relationships. There was also some flattening of the log dose-response relationship after icatibant, especially at the highest dose.

Discussion

The lack of effects of icatibant on heart rate or blood pressure accord with previous phase I studies (Collins, Hoechst UK, personal communication). The estimated values of $t_{1/2}$ are in good agreement with values of $t_{1/2\alpha}$ in these earlier studies. The finding that icatibant causes potent and dose related inhibition of bradykinin-induced vasodilation in the human forearm accords with this being a B_2-receptor mediated effect. Flattening of the bradykinin log dose-response curve following the highest dose of icatibant could be taken as evidence of a non-competitive component of the action of icatibant, but should not be over-interpreted: it is possible that some degree of desensitisation [10] occurred at the highest doses of bradykinin during the prolonged cumulative dose-response

studies, which would reduce the slope of the dose/response relationship. Conversely, plasma icatibant concentration was falling during the time taken to obtain the dose/response data, which will have the opposite effect.

These results demonstrate that intravenous icatibant antagonises bradykinin-induced forearm vasodilation in a dose-dependent manner and extends the findings of previous *in vitro* studies [21] to a human vascular bed *in vivo*. Mean plasma concentrations of icatibant when the bradykinin infusions were started were 9.5 ± 0.6 and 46.4 ± 2.0 nmol l^{-1} following the lowest and highest doses of icatibant, each dose causing significant inhibition of the effects of exogenous bradykinin. The pA_2 of icatibant in guinea pig ileum is 8.42 [21]. Thus the potency of icatibant is comparable in human vasculature *in vivo* and guinea-pig ileum *in vitro*.

The lack of effect of icatibant on blood pressure, heart rate or basal forearm blood flow (before infusion of bradykinin, and in the control arm throughout each study) suggests that under basal physiological conditions bradykinin does not play an important role in the maintainence of vascular tone in healthy young adult men. Bradykinin may play a role in a number of pathological conditions including allergic rhinitis, asthma, inflammatory bowel disease, pancreatitis and septic shock [3]. It has also been suggested that abnormal kinin status is of functional importance in hypertension. In rat models of hypertension studies have shown both reduced urinary excretion of tissue kallikrein and also increased inactivation of circulating kinins [23, 24]. Decreased urinary excretion of tissue kallikrein has also been reported in human hypertension, and hog pancreatic kallikrein administered orally to hypertensive patients not only corrects decreased urinary excretion of kallikrein but also lowers blood pressure [25].

ACE is a dipeptidyl peptidase with a variety of potential substrates including bradykinin [26] in addition to angiotensin I. Local ACE inhibition not only blocks the constrictor effect of angiotensin I but also enhances the dilator response to bradykinin in the human forearm [10, 27]. ACE inhibitors are effective in experimental and human hypertension in circumstances when circulating renin is low or normal [28–31], and it has been suggested that the hypotensive response to ACE inhibition may be mediated in part by potentiation of bradykinin either in the circulation or tissues [32]. Animal studies have shown that a bradykinin antagonist (B4147) that is less potent than icatibant can blunt the hypotensive response to ACE inhibition by approximately 40% [33].

In conclusion, icatibant is well-tolerated at doses that cause profound and dose-related inhibition of bradykinin-induced vasodilation in human forearm vasculature. Potency in this preparation is similar to that in guinea-pig ileum. Inhibition persists at least 1–15 h after discontinuing intravenous infusion of the doses used. These findings provide a quantitative basis for future studies of the role of bradykinin in the mechanism of action of ACE inhibitors, and in the pathophysiology of hypertension and other disorders. Furthermore, it is possible that B_2-receptor antagonists may also be used clinically in the future for treatment of conditions such as rhinitis, pancreatitis and septic shock where bradykinin has been implicated.

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