The effect of HN-65021 on responses to angiotensin II in human forearm vasculature

JOHN R. COCKCROFT, PHILIP J. CHOWIENCZYK, SALLY E. BRETT, TIMOTHY G. K. MANT, COLIN DURNIN¹, FRANCES LYNN¹, PAUL STEVENSON¹ & JAMES M. RITTER

Department of Clinical Pharmacology, UMDS St Thomas' Hospital, London, U.K. and ¹Hafslund Nycomed Pharma, Linz, Austria

We studied the effect of (2-butyl-4-chloro-1[[2'-(1H-tetrazol-5-yl) [1,1'-biphenyl]methyl]-1H-imadazole-5-carboxylic acid,-1-(ethoxycarbonyloxy) ethyl-ester (HN-65021), on angiotensin II induced vasoconstriction in forearm vasculature of eight healthy men. Placebo and HN-65021 (5, 10 and 100 mg) were administered orally. Forearm blood flow was measured by venous occlusion plethysmography during rising dose brachial artery infusions of angiotensin II (0.3–1000 pmol min⁻¹) 2 h after dosing. HN-65021 inhibited angiotensin II, causing a shift to the right of the dose-response curve. Angiotensin II (100 pmol min⁻¹) decreased mean blood flow in the infused arm by 63.1 ± 3.2% when infused following placebo and by 49.9 ± 4.3%, 50.7 ± 3.5% and 36.4 ± 2.8% following HN-65021 doses of 5, 10 and 100 mg respectively. These results demonstrate that HN-65021 antagonises angiotensin II receptor mediated vasoconstriction in human forearm resistance vessels.

Keywords forearm blood flow angiotensin II angiotensin receptors

Introduction

Angiotensin converting enzyme (ACE) inhibitors provide a practical means of inhibition of the renin angiotensin system and these drugs have become well established in the treatment of essential hypertension and heart failure [1, 2]. However converting enzyme is not specific for angiotensin I (ANG I), and in addition to preventing the conversion of ANG I to angiotensin II (ANG II), ACE inhibits breakdown of endogenous vasodilators such as bradykinin [3]. Accumulation of kinins may contribute to side effects associated with ACE inhibition such as cough [4] and angioedema [5]. ANG II receptor blockade does not cause kinin accumulation, resulting in distinct profiles of pharmacological activity of angiotensin receptor antagonists and ACE inhibitors [6].

HN-65021 is an orally active non-peptide angiotensin II AT₁ receptor antagonist pro-drug which is rapidly metabolized to the active moiety HN-12206. HN-65021 is presently being assessed for treatment of hypertension. We have studied the pharmacological action of HN-65021 in the human forearm vascular bed by measuring vasoconstrictor responses to intra-arterial ANG II following oral administration of HN-65021 or placebo.

Methods

Subjects

Experiments were performed in eight healthy male volunteers aged 23–28 years who gave written informed consent to take part in the study, which was approved by the Lewisham and North Southwark ethics committee. No clinically significant abnormalities were detected on routine biochemical and haematological screening. Subjects maintained their usual diet and salt intake during the study period.

Protocol

Subjects were studied on four occasions, 1 week or more apart receiving placebo or HN-65021 5, 10 and 100 mg orally according to a randomized, double-blind cross-over design. On each study day heart rate and blood pressure were measured 30 min before dosing, at dosing, at hourly intervals for 4 h and after conclusion of the blood flow study.

The left brachial artery was cannulated and forearm blood flow measured in both arms simultaneously using venous occlusion plethysmography as previously described [6]. Following the determination of stable

baseline flow stepwise increasing doses of ANG II (0.3, 3, 30, 100, 300, 1000 pmol min⁻¹, each dose for 6 min) were infused 2 h after the start of dosing with HN-65021 until an estimated reduction in forearm blood flow from baseline of 50–60% was achieved [6].

Blood samples were collected via an intravenous cannula in the non-infused arm for measurement of HN-12206 by high performance liquid chromatography every 30 min until 2 h post dosing and then at 3, 4, 6, 8, 12 and 24 h.

Statistical analysis

Data are summarized as means $\pm$ s.e. mean. Statistical analysis was by repeated measures analysis of variance with pairwise comparisons. Differences were considered significant if $P < 0.017$ (Bonferroni correction).

Results

Blood pressure, heart rate and forearm blood flow in each arm during saline infusions did not differ significantly between the 4 study days. Blood pressure immediately after the forearm blood flow study was $125 \pm 4/57 \pm 3$ mmHg on the placebo day and $126 \pm 4/58 \pm 2$ mmHg on the day on which the maximal dose of HN65021 was given (95% confidence intervals for the difference: -6 to $+6$ and -5 to $+5$ mmHg for systolic and diastolic pressures respectively). ANG II produced a dose-dependent decrease in blood flow in the infused arm on all study days without any subjective sensation. There were no serious drug related adverse effects.

HN-12206 concentrations

Plasma concentrations of the active moiety HN-12206 following oral administration of HN-65021 reached a maximum 1–2 h post dosing. HN-12206 was quantifiable in all subjects 2 h after each dose: 33.7 ± 2.8 , 55.7 ± 6.3 and 399.0 ± 73.9 ng ml⁻¹ respectively. After the 5 mg dose the plasma concentration was quantifiable in all subjects up to 2 h. After the 10 mg dose HN-12206 was quantifiable in all subjects up to 6 h and after the 100 mg dose in all subjects up to 12 h. These results are shown in Figure 1.

Effect of HN-65021 on forearm blood flow responses to ANG II

ANG II produced a dose-dependent decrease in blood flow in the infused arm on all study days. HN-65021 inhibited responses to ANG II, ($P < 0.01$ for all doses), shifting a logarithmic plot of the dose-response relationship to the right (Figure 2). ANG II (100 pmol min⁻¹) reduced mean blood flow in the infused arm by $63.1 \pm 3.2\%$, $49.9 \pm 4.3\%$, $50.7 \pm 3.5\%$ and $36.4 \pm 2.8\%$

Figure 1 Plasma concentrations of HN-12206 (ng ml⁻¹) mean $\pm$ s.e. mean following oral administration of HN-65021 10 mg (open squares) and 100 mg (closed squares).

Figure 2 Percentage reduction in forearm blood flow (mean $\pm$ s.e. mean) produced by brachial artery infusion of angiotensin II following oral administration of placebo (closed squares), HN-65021 5 mg (open squares), 10 mg (open circles) and 100 mg (open triangles).

following placebo, 5, 10 and 100 mg HN-65021 respectively. After HN-65021 100 mg a thirtyfold higher dose of ANG II (300 pmol min⁻¹) was needed in order to achieve a similar reduction in blood flow ($43.7 \pm 3.5\%$) to that produced by 10 pmol min⁻¹ of ANG II following placebo ($43.8 \pm 3.2\%$).

Discussion

The lack of effect of HN-65021 on heart rate, blood pressure or basal forearm blood flow is expected in healthy normotensive salt-replete subjects [6, 7]. The results demonstrate that oral HN-65021 antagonizes ANG II forearm vasoconstriction in a dose dependent manner. The potency of HN-65021 is similar to that of losartan another ANG II receptor antagonist in this vascular bed [6].

ANG II can be generated by non-ACE mediated mechanisms such as the chymase pathway [8] and can be detected in plasma and tissues despite ACE inhibition [9]. HN-65021 may provide more complete inhibition of the renin-angiotensin system than ACE inhibitors.

ANG II receptor antagonism, unlike ACE inhibition, does not potentiate bradykinin induced vasodilation [6]. Bradykinin accumulates in plasma and tissues during treatment with ACE inhibitors [10, 11] and may contribute to some of the clinically important side-effects of ACE inhibition [12]. Cough has emerged as one of the most common class effects of ACE inhibitor therapy with reported incidence varying between 1 and 30% [13]. As cough is believed to result from inhibition of ACE rather than blockade of ANG II production *per se*. ANG II receptor antagonists may be free of this troublesome side-effect. Indeed a recent trial has shown that the incidence of cough during treatment with the ANG II receptor antagonist losartan is lower than that observed with the ACE inhibitor lisinopril in patients with ACE inhibitor cough challenged with these agents [14].

In conclusion, these studies demonstrate that well tolerated doses of HN-65021 inhibit ANG II-induced vasoconstriction in human forearm vasculature. These data provide a pharmacodynamic basis for the further investigation of HN-65021 for the treatment of conditions associated with undesired activation of the renin angiotensin system including hypertension and congestive heart failure.

This study was supported by Hafslund Nycomed Pharma AG and by a contribution from the Austrian Industrial Research Promotion Fund.

References

- 1 Frohlich ED. Angiotensin converting enzyme inhibitors: present and future. *Hypertension* 1989; **13**: 125–130.
- 2 Dargie HJ, McMurray JJV. Diagnosis and management of heart failure. *Br Med J* 1994; **308**: 321–328.
- 3 Dorer FE, Kahn JH, Lentz KE, Levine M, Skeggs LT. Hydrolysis of bradykinin by angiotensin converting enzyme. *Circ Res* 1974; **34**: 824–827.
- 4 Webb DJ, Benjamin N, Collier J, Robinson B. Enalapril-induced cough. *Lancet* 1986; **ii**: 1094.
- 5 Slater EE, Merrill DD, Guess HA, Roylance PJ, Cooper WD, Inman WHW, Ewan PW. Clinical profile of angioedema associated with angiotensin converting enzyme inhibition. *JAMA* 1988; **260**: 967–970.
- 6 Cockcroft JR, Sciberras DG, Goldberg MR, Ritter JM. Comparison of angiotensin-converting enzyme inhibition with angiotensin II receptor antagonism in the human forearm. *J Cardiovasc Pharmacol*; **22**: 579–584.
- 7 Vidt DG. A controlled multiclinic study to compare the effects of MK421, hydrochlorothiazide and MK421 combined with hydrochlorothiazide in patients with mild to moderate essential hypertension. *J Hypertens* 1984; **2** (suppl 2): 81–88.
- 8 Husain A. The chymase-angiotensin system in humans. *J Hypertens* 1993; **11**: 1155–1159.
- 9 Mento PF, Wilkes BM. Plasma angiotensins and blood pressure during converting enzyme inhibition. *Hypertension* 1987; **9** (suppl III): III42–III48.
- 10 Pellacani A, Brunner HR, Nussberger J. Plasma kinins increase after angiotensin-converting enzyme inhibition in human subjects. *Clin Sci* 1994; **87**: 567–5574.
- 11 Campbell DJ, Kladis A, Duncan A-M. Effects of converting enzyme inhibitors on angiotensin and bradykinin peptides. *Hypertension* 1994; **23**: 439–449.
- 13 Lindgren BR, Andersson RGG. Angiotensin-converting enzyme inhibitors and their influence on inflammation, bronchial reactivity and cough: a research review. *Med Toxicol Advers Drug Exp* 1989; **4**: 369–380.
- 14 Lacourciere Y, Brunner H, Irwin R, *et al.* and the Losartan Cough Study Group. Effects of modulators of the renin-angiotensin-aldosterone system on cough. *J Hypertens* 1994; **12**: 1387–1393.

(Received 10 March 1995,
accepted 9 August 1995)