A dose-response study of HOE 498, a new non-sulphydryl converting enzyme inhibitor, on blood pressure, pulse rate and the renin-angiotensin-aldosterone system in normal man

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1 The effect of different oral doses of HOE 498, a new non-sulphydryl containing converting enzyme inhibitor, was investigated in a double-blind, placebo-controlled study in normotensive volunteers.

2 Dose-related reductions in serum converting enzyme activity, plasma angiotensin II and aldosterone were seen, greater at 4 h than at 12 h after drug ingestion. Converse dose-related increases in blood angiotensin I and plasma active renin concentration occurred.

3 Falls of angiotensin II were as great with 20 mg as with 50 mg of HOE 498, although the effect was more prolonged with 50 mg.

4 The reductions in concentrations of plasma angiotensin II and serum converting enzyme activity and the increases in plasma renin concentration were correlated with the concentration of HOE 498 - diacid in plasma.

5 Dose-related falls in both supine and erect blood pressure were maximal 2-3.5 h after dosing. Pulse rate increased marginally but insignificantly in the supine; slightly and significantly in the upright position, concomitantly with the blood pressure reduction at all doses of active drug.

6 We conclude that effects of single doses of HOE 498 on the renin-angiotensin system are maximal within 4 h, but are still apparent after 24 h. Thus it is likely that once daily administration will be adequate for treatment of high blood pressure in patients.

Keywords converting enzyme inhibition HOE 498 blood pressure renin-angiotensin

Introduction

Angiotensin converting enzyme (ACE) inhibitors have proved effective therapy for hypertension (Atkinson & Robertson, 1979; Brunner *et al.*, 1979; Hodsman *et al.*, 1983a) and heart failure (Atkinson & Robertson, 1979; Dargie *et al.*, 1983; Wenting *et al.*, 1983). Captopril, the first commercially available ACE-inhibitor, possesses some toxicity probably related in part to its sulphydryl group. Moreover, captopril requires at least twice daily administration. The

introduction of longer acting non-sulphydryl containing ACE-inhibitors, with potentially less toxicity (Dollery, 1983), seems likely to widen the indications for ACE-inhibitors (Ball & Robertson 1985).

In the present placebo-controlled study we report the effects of three single doses of HOE 498, a new orally active, non-sulphydryl containing ACE-inhibitor (Becker *et al.*, 1983; Witte *et al.*, 1983) (Figure 1) on blood pressure

(BP), pulse rate (PR), and components of the renin-angiotensin-aldosterone system in normotensive volunteers.

Captopril

Enalapril

HOE 498

Figure 1 Structures of captopril, enalapril (MK421) and HOE 498.

Methods

Study population

Twelve healthy, normotensive volunteers aged 21–30 years, weighing between 65–80 kg (mean 70.1 ± 1.4 kg) were included in the study which was approved by the Ethics Supervisory Committee at the Western Infirmary, Glasgow. The nature of the experiment was explained to each volunteer and written consent was obtained. Each subject had a medical history taken and a physical examination performed. None had a family history of hypertension. Routine laboratory investigations and electrocardiography were performed before administration of the drug and were normal. Throughout the investigation, the subjects were maintained on a free regular salt intake.

Design of the study

Each subject was investigated on four different occasions at the same time of the day, not less than 1 week apart. On the morning of the study the subjects, after fasting overnight, attended the ward and lay supine at 08.00 h. Blood pressure (Hawksley random sphygmomanometer) was then measured in both arms

and the arm with the higher readings was used for further measurements. Phase V was taken as diastolic blood pressure. Mean arterial pressure (MAP) was taken as diastolic plus one-third of the pulse pressure. The same observer (PJOM) measured blood pressure throughout. A cannula was inserted into an antecubital vein for blood sampling.

Blood samples were drawn before (08.45–09.00 h) and throughout the 24 h (13.00, 21.00 and 09.00 h the next day) after drug administration. Blood pressure and pulse rate were measured with the arm supported at heart level both supine and after 2 min standing. The drug was given at 09.00 h following which the subjects ate a light breakfast. They were confined to bed until 13.00 h, with supine and erect (2 min standing) blood pressure and pulse rate measurements half-hourly until 12.00 h.

Further blood pressure and pulse rate recordings were taken prior to blood sampling at 4, 12 and 24 h after the drug or placebo administration. The subjects continued a routine day except that they were required to lie down for 1 h before these measurements. They again fasted overnight for a further sample 24 h from dosing.

Medication

Capsules containing 5 mg, 20 mg and 50 mg of HOE 498 or placebo were supplied by Hoechst Pharmaceutical, Hounslow, Middlesex, U.K. The subjects received a single oral dose with 150 ml water at 09.00 h on the day of study. The sequence of drug administration was randomised according to a Latin square design. The study was double-blind.

Analytical methods

Plasma active renin concentration (normal range 10–50 mu/l, inter-assay coefficient of variation (CV) 11%) was determined by the method of Millar *et al.*, (1980); blood angiotensin I (normal range 3–15 pmol/l, inter-assay CV 14.9%) according to Waite (1973) and Morton *et al.* (1976); plasma angiotensin II (normal range 3–35 pmol/l, inter-assay CV 10%) according to Morton *et al.* (1976) and Atkinson *et al.* (1980); plasma aldosterone (normal range < 500 pmol/l, inter-assay CV 17%) according to Fraser *et al.* (1973); and serum converting enzyme activity (ACE) (normal range 1.1–1.7 $\mu\text{mol hippurate ml}^{-1} \text{ h}^{-1}$, inter-assay CV 8.2%) according to Lieberman (1975). This last measures the rate of degradation of a tripeptide substrate. The concentration of the

diacid of HOE 498 in serum was measured according to the method of Hajdu *et al.* (1984) (limit of detection 0.05 ng/ml; inter-assay CV 7%). Basal values of angiotensin II are the means of the values of two samples drawn at 08.45 and 09.00 h respectively; these two resting samples for angiotensin II were taken as part of another study. In all other instances a single basal blood sample was used for the analysis.

Statistical evaluation

The data were evaluated using repeated measures analysis of variance, and the results presented (Tables 2 and 3) are the follow up multiple comparisons which are based on paired *t*-tests. Logarithmic transformations of the data were used where appropriate. Each *t*-test compared the changes on one dose of HOE 498 with the changes on placebo. One test, for example, compared the change in supine systolic blood pressure from 0 to 4 h on 5 mg HOE 498 with the corresponding change on placebo. Significant results are stated either as being significant at the 5% level as an individual comparison (*) or as being significant at the 5% level after making a Bonferroni correction to allow for the 9 comparisons which were made on each variable (**) (Miller, 1966). The basal values taken in this part of the analysis are those at 09.00 h except for angiotensin II where, as mentioned above, the mean of the 08.45 and 09.00 h results was used.

A separate follow-up analysis was performed for the multiple blood pressure and pulse rate measurements from the first 4 h after drug intake (Table 1 and Figure 2 only). For each subject in this analysis the mean of the 09.00 and 09.30 h blood pressure and heart rate values was calculated and compared with the mean of all subsequent values until 13.00 h; this method of analysis was agreed before beginning the study. As above, changes on the active drug were compared with the corresponding changes on placebo, and a Bonferroni correction was used to allow for the three comparisons which were made on each variable.

Results

Blood pressure and pulse rate

HOE 498 significantly reduced supine and erect mean arterial blood pressure (MAP) (Figure 2). Maximal blood pressure fall was seen in individual subjects 2–3.5 h after drug intake. Pulse rate was not significantly increased in the supine

posture (greatest change from 60.2 ± 2.7 s.e. mean to 64.8 ± 3.5 beats/min 1.5 h after 50 mg). However heart rate was slightly and significantly raised in the upright position, concomitantly with the blood pressure reduction during the first four hours after all doses of active drug (Table 1). These early changes in heart rate and blood pressure were seen even though the method of analysis (employing the mean of 09.00 and 09.30 h values as basal) would have tended to minimize the statistical significance.

Figure 2: Supine and erect mean arterial blood pressure (MAP) for 4 h following intake of HOE 498 (5 mg, ▲; 20 mg, ○; 50 mg, ■) and placebo (----). For clarity, standard errors have been omitted. Supine MAP was significantly reduced after HOE 498 5 (*), 20 (**), and 50 mg (**), erect MAP after 20 (**) and 50 mg (**).

All three doses of HOE 498 maintained a reduced supine MAP at 4 h after drug intake, but the decrease was small; a significant fall was not seen consistently at 12 and 24 h (Table 2). Erect blood pressure was again reduced later than 4 h after drug administration but the changes were small and failed to reach significance at most time points (Table 2). No consistent changes in

Table 1 Erect pulse rate (beats/min) for 4 h after intake of HOE 498 and placebo. The pulse rate was significantly increased after HOE 498 5 mg (*), 20 mg (**) and 50 mg (**). Mean $\pm$ s.e. mean.

	Time (h)							
	0.00	0.5	1.00	1.5	2.00	2.5	3.00	4.00
Placebo	73.7 ± 3.7	76.7 ± 3.6	74.5 ± 4.6	75.7 ± 4.8	72.5 ± 4.3	73.0 ± 4.4	71.5 ± 4.5	71.7 ± 4.2
5 mg HOE 498	75.7 ± 2.9	73.8 ± 3.1	76.2 ± 3.1	80.0 ± 3.0	81.3 ± 3.8	82.3 ± 3.5	76.8 ± 2.3	72.8 ± 2.9
20 mg HOE 498	79.3 ± 5.0	80.0 ± 4.4	80.8 ± 4.8	86.8 ± 5.3	85.3 ± 6.1	86.2 ± 5.8	82.2 ± 5.9	82.8 ± 6.4
50 mg HOE 498	75.0 ± 3.9	74.2 ± 3.3	75.7 ± 3.3	81.2 ± 3.9	78.2 ± 4.5	82.5 ± 3.2	83.3 ± 3.7	78.0 ± 3.8

supine or erect pulse rates were observed 4, 12 and 24 h after intake of HOE 498 (Table 2).

Converting enzyme activity

Serum converting enzyme activity was reduced markedly after administration of HOE 498 at all doses of active drug (Table 3, Figure 3). The reduction was well-sustained for up to 24 h after drug intake.

Active renin, angiotensin I and angiotensin II

Circulating concentrations of active renin and angiotensin I rose, and angiotensin II fell, in a dose-related fashion (Table 3, Figure 3). The greatest changes were seen 4 h after dosing but were still partly evident at 24 h.

Aldosterone

Plasma aldosterone levels were rather low in the basal state (Table 3, Figure 3). Even so, reductions in aldosterone concentrations were found after drug administration, with the nadir rather later than that of angiotensin II, but these decreases did not always achieve significance.

Plasma concentration of the diacid of HOE 498

The bioactive metabolite of HOE 498, a diacid, was measurable in all the plasma samples of volunteers having active drug. The plasma concentration increased significantly with increasing dosages (Figure 4), with highest values being recorded after 4 h. Measurable, but low, amounts were still found 24 h after intake of HOE 498. For each subject there were usually close relationships between the 16 individual

measurements of plasma concentrations of HOE 498-diacid and circulating concentrations of converting enzyme activity (range $r = -0.81$ to -0.96 , mean -0.91), angiotensin II (range $r = -0.45$ to -0.83 , mean -0.68) and active renin (range $r = +0.08$ to 0.93 , mean 0.69).

Side-effects

No serious untoward effects were encountered. One subject felt faint 4 h after placebo and one subject 12 h after receiving HOE 498, 50 mg.

Discussion

HOE 498 is a new orally active and potent inhibitor of ACE in plasma and various tissues of animals (Becker *et al.*, 1983) (Figure 1). HOE 498 itself is a non-sulphydryl-containing prodrug, which after absorption is rapidly converted to its active metabolite, a diacid, in the liver. HOE 498 exerts potent antihypertensive effects in experimental hypertension in rodents and dogs (Schölkens *et al.*, 1983). Witte *et al.* (1983) administered the drug for the first time to man in dosages of up to 3.2 mg but could not detect lowering of blood pressure in normotensive volunteers.

In the present study, blood pressure was lowered by active drug up to 24 h after dosing, the most consistent reduction being achieved after 2–3 h. The reduction of blood pressure after 5–50 mg of HOE 498 was comparable to that seen after enalapril and lisinopril in the dose range 5–20 mg, at 4–6 h after intake (Hodsman *et al.*, 1984; MacGregor *et al.*, 1981a; Millar *et al.*, 1982) or 2 h after 25 mg of captopril (MacGregor *et al.*, 1981b). The time curve of blood pressure

Table 2 Supine and erect mean arterial blood pressure (MAP) (mmHg) and supine and erect pulse rate (beats/min) at 0, 4, 12 and 24 h after intake of HOE 498 and placebo. Mean $\pm$ s.e. mean. Paired *t*-test was performed comparing changes from basal on placebo with corresponding changes on active drug.

	Placebo				5 mg				20 mg				50 mg			
	Basal	4 h	12 h	24 h	Basal	4 h	12 h	24 h	Basal	4 h	12 h	24 h	Basal	4 h	12 h	24 h
Supine MAP	85.9 ± 2.3	89.8 ± 1.9	90.9 ± 3.0	85.5 ± 2.2	88.6 ± 2.9	88.8* ± 2.3	86.2* ± 3.0	83.8* ± 2.4	89.9 ± 1.8	83.2** ± 2.4	88.8 ± 2.7	84.9* ± 1.8	88.7 ± 1.4	84.9* ± 3.7	86.9* ± 2.0	83.8 ± 2.3
Supine Heart rate	59.1 ± 2.4	60.0 ± 3.2	67.2 ± 3.0	62.5 ± 1.9	59.7 ± 2.5	58.0 ± 1.9	64.5 ± 2.7	60.2 ± 2.8	63.0 ± 2.9	62.7 ± 3.4	64.7* ± 2.9	62.8 ± 3.1	60.2 ± 2.7	62.2 ± 2.9	68.7 ± 3.8	61.8 ± 2.2
Erect MAP	94.5 ± 1.6	95.9 ± 2.3	93.8 ± 2.7	91.2 ± 2.2	98.4 ± 2.8	93.1 ± 2.8	91.1* ± 2.9	88.7 ± 2.3	93.7 ± 1.9	89.1 ± 2.3	91.9 ± 2.7	87.6 ± 2.3	93.1 ± 1.8	87.7* ± 3.5	90.6 ± 1.4	87.6 ± 2.1
Erect Heart rate	73.7 ± 3.7	71.7 ± 4.3	86.5 ± 3.9	83.0 ± 2.7	75.7 ± 2.9	72.8 ± 2.9	87.5 ± 3.8	82.0 ± 3.5	79.3 ± 5.1	82.8 ± 6.5	87.3 ± 5.0	83.7 ± 4.5	75.0 ± 3.9	78.0 ± 3.9	85.8 ± 3.8	79.2 ± 4.4

* $P < 0.05$ as an individual test, ** $P < 0.05$ after making a Bonferroni correction due to multiple comparisons on each variable.

response to HOE 498 was similar to that seen after enalapril and lisinopril and suggests that HOE 498 might also be used in once daily administration to treat hypertensive patients.

The lowering of blood pressure seen here confirms earlier suggestions that circulating plasma concentrations of angiotensin II are within a range having a direct effect on blood pressure in normotensive salt-replete man (Chinn & Düsterdieck, 1972; Atkinson & Robertson, 1979; Niarchos *et al.*, 1979; MacGregor *et al.*, 1981b).

The effect of HOE 498 on plasma angiotensin II was seen to be more prolonged than that of captopril (MacGregor *et al.*, 1981b) and of similar duration to that of enalapril (Brunner *et al.*, 1981; MacGregor *et al.*, 1981a; Hodsman *et al.*, 1984) and lisinopril (Hodsman *et al.*, 1984).

In contrast to earlier studies with ACE-inhibitors such as captopril (MacGregor *et al.*, 1981b) enalapril and lisinopril (Hodsman *et al.*, 1984; Millar *et al.*, 1982) we observed a small increase in pulse rate concurrently with the blood pressure reduction. Greater increases in heart rate are occasionally observed with the clinical use of both captopril (Atkinson *et al.*, 1982) and enalapril (Hodsman *et al.*, 1983b).

Because a fall in plasma angiotensin II causes peripheral vasodilatation, a rise in heart rate with converting enzyme inhibition is not unexpected. Vasodilators such as hydralazine, which act mostly on resistance vessels, cause a much more pronounced pulse rate increase in response to a given blood pressure fall (Åblad, 1959; Wilkinson *et al.*, 1952). Our finding of a very modest pulse rate increase is more like the effect of those vasodilators which act on both resistance and capacitance vessels (Kaneko *et al.*, 1967).

However, angiotensin II has a range of other effects, including sympathetic stimulation and vagal inhibition (Scroop & Lowe, 1969; Lumbers *et al.*, 1979; Lee *et al.*, 1980). Although neither captopril (Manhem *et al.*, 1981) nor enalapril (Millar *et al.*, 1982) measurably affect plasma noradrenaline levels, they, like HOE 498, might well limit compensatory pulse rate changes by withdrawal of vagal inhibition.

Thus single doses of 5, 20 or 50 mg HOE 498 have their effect on the renin-angiotensin system at or before 4 h from administration. The action is still apparent after 24 h and is more sustained at higher doses. This suggests that once daily administration of HOE 498 will be adequate for the treatment of high blood pressure in patients.

Table 3 Serum converting enzyme activity (ACE) (μmol hippurate $\text{ml}^{-1} \text{h}^{-1}$), plasma active renin concentration (muI/l), blood angiotensin I (pmol/l), plasma angiotensin II (pmol/l), the ratio of angiotensin I to angiotensin II (units) and plasma aldosterone (pmol/l) immediately before and 4, 12 and 24 h after intake of HOE 498 5, 20 and 50 mg or placebo. Mean $\pm$ s.e. mean. For level of significance—see text to Tables 1 and 2.

	Placebo				5 mg				HOE498 20 mg				50 mg			
	Basal	4 h	12 h	24 h	Basal	4 h	12 h	24 h	Basal	4 h	12 h	24 h	Basal	4 h	12 h	24 h
ACE	1.29 ±0.04	1.29 ±0.05	1.30 ±0.04	1.29 ±0.04	1.27 ±0.06	0.12** ±0.02	0.31** ±0.02	0.5** ±0.02	1.27 ±0.04	0.05** ±0.01	0.25** ±0.03	0.44** ±0.03	1.25 ±0.04	0.03** ±0.01	0.1** ±0.02	0.28** ±0.03
Renin	26.1 ±4.4	23.6 ±3.9	32.2 ±5.2	36.3 ±5.5	30.4 ±6.4	60.0* ±14.9	72.8* ±13.9	57.2* ±6.2	32.1 ±6.5	150.8** ±29.4	113.6* ±34.4	77.3** ±11.8	28.4 ±5.6	209.8** ±78.5	180.2** ±42.2	104.8** ±23.3
AI	8.6 ±1.2	6.8 ±0.9	8.0 ±1.1	9.3 ±1.4	9.3 ±1.8	25.2** ±4.4	26.3** ±5.5	23.0** ±2.9	8.7 ±0.9	50.5** ±11.3	43.0* ±13.4	30.2** ±6.6	7.9 ±1.3	88.0** ±38.4	67.5** ±26.2	34.4** ±6.7
AII	10.7 ±1.1	7.4 ±0.9	9.0 ±1.8	11.2 ±1.8	12.9 ±1.8	4.7* ±0.7	6.2 ±0.8	9.8 ±1.4	12.1 ±2.4	2.9** ±0.3	6.7 ±1.3	10.7 ±2.0	12.3 ±2.0	2.8** ±0.3	4.3 ±0.4	9.4 ±1.5
AI/ AII	0.8 ±0.1	1.0 ±0.2	1.1 ±0.2	0.9 ±0.2	0.8 ±0.1	6.9** ±1.9	4.3** ±0.8	2.5** ±0.4	0.9 ±0.1	20.6** ±5.7	10.4* ±5.2	2.9** ±0.5	0.7 ±0.1	66.2** ±39.6	16.7** ±4.8	3.6** ±0.5
Aldosterone	173 ±85	179 ±132	149 ±107	242 ±118	228 ±124	127** ±85	107 ±66	198** ±83	212 ±132	113* ±91	110 ±85	209 ±146	215 ±102	171 ±124	94* ±74	239 ±138

Figure 3 Mean changes in components of the renin-angiotensin system after HOE 498 5 mg (▲), 20 mg (○), 50 mg (■) and placebo (----). For clarity, standard errors have been omitted. ACE denotes serum converting enzyme activity.

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Figure 4 Concentration (mean $\pm$ s.e. mean) of the diacid of HOE 498 after intake of 5 mg (▲), 20 mg (○), 50 mg (■) HOE 498 and placebo (----).

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