STUDIES OF THE CLINICAL PHARMACOLOGY OF PERINDOPRIL

A new inhibitor of angiotensin converting enzyme

A thesis by

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DECLARATION

The work described in this thesis was carried out while I was employed as a registrar in the Department of Materia Medica. I enjoyed collaboration with Dr L A Ajayi for the study on autonomic function after perindopril. He undertook several of the tests of autonomic reflexes. The direction and coordination of the laboratory analyses was shared between myself and Dr PA Meredith. The remainder of the work and all of the statistical analysis was carried out by myself. The writing of this thesis was entirely my own work.

SUMMARY

SUMMARY

Over the last thirty years, effective and relatively safe control of hypertension has become possible. As a result, less severe forms of high blood pressure now warrant treatment. Such treatment needs to be acceptable to the patient and free from long-term toxicity.

The angiotensin converting enzyme (ACE) inhibitors offer the possibility of improved tolerability and a novel mechanism of action. After review of the renin-angiotensin system, the clinical pharmacology of the two currently available ACE inhibitors is discussed. Captopril and enalapril have been demonstrated to be effective treatments in hypertension and cardiac failure. Both drugs may cause significant side effects and so there is justification for investigation of a similar compound which has an excellent safety profile in laboratory studies.

The steps involved in the transfer of drugs from the animal laboratory to early clinical studies in man are then discussed.

Perindopril is the esterified form of a potent and long-acting ACE inhibitor, S-9780: it is a prodrug which relies on bioactivation in vivo. The studies described in this thesis include some of the earliest administrations of perindopril to man and the first clinical trial involving S-9780.

In the earliest dose-ranging study, 36 normotensive volunteers, in parallel groups of 6 subjects, were given perindopril (1 to 16 mg)

orally for 7 consecutive days. The drug was well tolerated and no sign of toxicity was detected. Blood pressure was lowered by active treatment (14/11 mmHg 6 hours after 16 mg) with only a slight rise in heart rate (15 beats.min^{-1}) after chronic treatment with 16 mg. Plasma ACE was inhibited and plasma renin activity was elevated in a dose-related pattern. Plasma aldosterone levels fell. The maximum effect occurred after 4 - 6 hours and 60% inhibition of plasma ACE persisted 24 hours after dosing with 8 and 16 mg. Plasma catecholamines were unchanged.

A double blind crossover study in 8 normotensive volunteers demonstrated that intravenous administration of the active metabolite of perindopril, S-9780, was well tolerated and apparently safe. Maximal inhibition of plasma ACE occurred after only 1 mg. A dose-related rise in plasma renin activity occurred but no effect on plasma aldosterone was detected. Blood pressure was lowered by 8/14 mmHg 3 hours after 4 mg of S-9780 intravenously with no change in heart rate or plasma catecholamines.

The effect of perindopril on autonomic function was assessed in a double blind, placebo controlled, crossover study in 10 normotensive males. Eight milligrammes given orally lowered blood pressure without a change in heart rate. Perindopril enhanced the vagally mediated heart rate variation with deep breathing. There was no impairment of the response to either bicycle exercise at 175 W for 5 minutes or isometric handgrip. The pressor response to cold and the response to the Valsalva manoeuvre were unaltered. These results suggested that the absence of tachycardia after perindopril might be in part related to enhanced parasympathetic tone.

In a single blind, placebo controlled study in seven hypertensive patients treated for one month, tolerability was excellent. Blood pressure was lowered from 164/93 mmHg to 145/84 mmHg by 4 mg of perindopril and after one month remained 142/82 mmHg. Neither postural hypotension nor tachycardia occurred. The biochemical effects were comparable to those in the volunteers.

Plasma S-9780 levels were determined by an enzyme inhibition assay following treatment with oral perindopril and intravenous S-9780. The kinetics were linear and were not altered by repeated dosing. The intravenous data showed triphasic decay with a terminal half-life of over 30 hours; despite this, the accumulation half-life after repeated dosing was under 9 hours. The controversy over the pharmacokinetics of ACE inhibitors is acknowledged. Bioavailability of S-9780 after perindopril given orally was 15%. The kinetics of S-9780 in man corresponded to the kinetics in the laboratory animals which were used for the preclinical studies.

Plasma ACE inhibition was related to plasma drug concentrations following intravenously administered S-9780: a close correlation was obtained using the Hill equation to describe the relationship. The plasma concentration of S-9780 which produced 50% inhibition of plasma ACE was 1.8 ± 0.9 $ng.ml^{-1}$.

After oral dosing, the peak effect on ACE lagged behind peak plasma S-9780 concentration by several hours. A concentration-effect model allowed description of this relationship. Using the parameters of the model, it was shown that the sensitivity of plasma ACE to S-9780

diminished slightly with repeated dosing. More rapid onset and offset of action also occurred.

In conclusion, perindopril was well tolerated by over 50 subjects treated for up to one month, with no evidence of toxicity. The diacid metabolite, S-9780, is a potent and long acting inhibitor of plasma ACE in humans, with predictable and dose-related effects on the renin-angiotensin system. Perindopril possesses useful blood pressure lowering activity without causing postural hypotension or tachycardia. It enhances parasympathetic activity: this latter property is probably a feature of all ACE inhibitors.

The kinetics of S-9780 are linear. The decay in plasma levels is triphasic with a terminal half-life in excess of 30 hours. Despite this, accumulation of S-9780 does not occur after 43 - 60 hours during repeated oral dosing. The bioavailability of S-9780 is about 15% and the concentration which produces 50% inhibition of plasma ACE in man is 1.8 ± 0.9 $ng.ml^{-1}$. The concentration-effect relationship between S-9780 and ACE inhibition is complex following oral administration of perindopril. Changes occur after repeated dosing which suggest diminished sensitivity to the drug, probably as a result of induction of plasma ACE.

These early studies with perindopril reveal how investigations in normal volunteers can provide information about the likely response of hypertensive patients to new ACE inhibitors. The study in patients confirms the usefulness of the volunteer studies and demonstrates that perindopril is worthy of more extensive evaluation in essential hypertension.

CHAPTER 1

BACKGROUND AND SCOPE OF THE THESIS

This chapter deals firstly with the background to the treatment of high blood pressure, continues with a discussion of the drugs which are in current use, concentrating particularly on the angiotensin converting enzyme inhibitors, and then moves on to consideration of the early clinical investigation of new drugs. Finally, an outline is given of the chapters which follow: in these, the early clinical investigation of a new angiotensin converting enzyme inhibitor, perindopril, is described.

1.1 TREATMENT OF HYPERTENSION

This century has seen remarkable advances in the understanding and management of high blood pressure. The most major of these concern, firstly, the development of satisfactory noninvasive means of measuring the blood pressure; secondly, identification of the contribution of hypertension to morbidity and mortality; and finally, recognition that treatment of severe hypertension results in greatly reduced morbidity and mortality. Thus, since the late 1950's, clear benefits from treatment of malignant hypertension even with rather unsatisfactory antihypertensive agents have been acknowledged (18,87).

Over the following 30 years the advances have been less dramatic but

of far greater benefit to a greater number of patients. In 1967, the Veterans Administration Cooperative Study Group on Antihypertensive Agents' trial of the effects of treatment on morbidity in less severe hypertension (206) demonstrated striking benefit of treatment to patients with diastolic pressures of over 115 mmHg. The same trial later showed that the benefit extended down to levels of pressure between 90 and 114 mmHg (207) and that the potential benefit was related to the level of pretreatment blood pressure. Active treatment halved the hypertension-related mortality in this group and had an even more profound effect on the morbidity. The narrow selection criteria for recruitment to this study relative to the at-risk population and the failure to demonstrate a statistically significant reduction in coronary events were amongst the reasons that further trials were considered necessary.

The results of the Hypertension and Follow-up Program Cooperative Group's trial (101) clarified some outstanding points. The benefit of treatment of diastolic blood pressures which exceed 90 mmHg was shown to apply to the whole population rather than just middle aged males. A reduction in deaths due to acute myocardial infarction occurred in the more intensively treated 'stepped care' group, although a decrease in deaths due to noncardiovascular diseases does raise the question of whether closer medical supervision rather than improved control of blood pressure may have accounted for some of the difference. There is, however, strong support for these results from other studies.

The Australian Therapeutic Trial in Mild Hypertension confirmed the benefit of treatment to a group of patients aged 30 - 69 years with

diastolic blood pressures of 95 - 104 mmHg (11) but also showed that the benefit extended to include those patients aged 65 - 74 years (149). In the light of these and other trials' results, a combined meeting of the World Health Organisation and the International Society of Hypertension in 1982 endorsed guidelines (219) which include active treatment for patients who have diastolic blood pressures consistently above 95 mmHg and for those with pressures between 90 and 95 mmHg who have additional risk factors.

The situation is complicated, however, by two other factors. The first of these is that a small percentage benefit spread over the large majority of patients who have mild hypertension will result in the saving of many more lives than a large percentage benefit restricted to a limited group of patients who have severe hypertenson. Thus, whilst in epidemiological terms there is sound evidence that morbidity and mortality will be reduced by such a strategy, it is nevertheless clear that the majority of patients who will be treated for the remaining duration of their lives will derive no benefit from the intervention. The second factor is related to the first: since the initial reports of benefit from antihypertensive therapy, the available drug regimens have been revolutionised; the modern treatments are not yet free from side effects, however, and long term administration may yet reveal new problems with some agents which are currently thought to be satisfactory.

The epidemiologist would argue in favour of a general approach, using the best available regimens to control blood pressure in all identified at-risk groups. He would stress the need for screening

and preventive measures on a population basis. The physician is aware that he must balance an individual's risk profile against his response to treatment and possible side effects. The patient will inevitably and rightly wish to be involved in the decisions. For the clinical pharmacologist, the issue must be to aid in the development of new agents which have improved risk/efficacy profiles and to clarify the mechanisms of action and of toxicity of currently available drugs.

1.2 DRUGS USED IN THE TREATMENT OF HYPERTENSION

The preceding discussion has outlined the need for well tolerated, safe and effective antihypertensive drugs. The agents which are currently in use are now examined. They are considered under broad headings: the β-adrenoceptor antagonists, the diuretics, the vasodilators, the centrally acting drugs and the angiotensin converting enzyme inhibitors, although discussion of the last group is reserved for the following section of the chapter. For each group, the mode of action is described. Clinical features are compared and contrasted, both across and within the groups, with particular emphasis on the safety and tolerance of the drugs. The need for further antihypertensive drugs is justified.

β-adrenoceptor Antagonists (β-blockers)

General

Following the fortuitous discovery that the β-blockers lowered blood pressure, propranolol was confirmed as a useful antihypertensive agent in the 1960's (168). Many compounds have become available and these are now amongst the most widely used cardiovascular drugs.

Mode of Action

Adrenergic receptors were classified in to α and β sub-types by Alquhist in 1948 (1). Since then, the β-receptors have been further studied and found to comprise two types, i.e. β_1 and β_2. The β_1-receptors are found mainly in cardiac muscle, where they mediate the positive inotropic and chronotropic effects of the catecholamines, and in adipose tissue. The β_2-receptors are found in the bronchi, pancreas and peripheral vasculature: in this site they produce vasodilatation. To a certain extent, therefore, the mode of action of the β-blockers can be predicted from the receptor distribution and function.

Acute administration reduces heart rate and cardiac output (195). A compensatory initial rise in peripheral resistance prevents a large initial fall in blood pressure (86) but subsequent adjustment to a lower level of pressure appears to occur. The β-blockers which possess partial agonist activity may act differently: whereas the pure antagonists affect blood pressure by an action on cardiac receptors, pindolol directly lowers vascular resistance (190). There is some evidence that the β-blockers may act via central mechanisms (124). Finally, although β-blockers depress renin activity, there

does not appear to be any association between this action and their antihypertensive effect.

Efficacy

The β-blockers cause moderate and generally predictable falls in blood pressure, often indistinguishable from the effect of the thiazide diuretics (142). Once daily treatment is usually adequate.

Clinical Features

The β-blockers are not a homogeneous group. Partial agonism at β-receptors has already been mentioned as one additional feature of some β-blockers. Other separate features include membrane stabilising activity, relative selectivity for the β_1-receptor, hydrophilicity and hepatic metabolism.

Dose selection is aided when first pass metabolism does not occur. Hydrophilicity reduces the amount of drug which crosses the blood-brain barrier and thus minimises the risk of sedation and nightmares. Cardioselectivity confers relative freedom from bronchospasm (47) and easier reversal by bronchodilators, as well as reduced risk of a pressor response to smoking (201) and to hypoglycaemia (45). Membrane stabilising activity is probably not relevant at the doses used clinically. Sotalol does, however, possess class III antiarrhythmic activity. Partial agonism offers theoretical advantages when heart failure or peripheral vascular disease are present, but convincing evidence of its practical benefit is awaited.

The side effects of the β-blockers are well recognised.

Bronchospasm, cardiac failure, heart block and peripheral vascular disease are major contraindications as each can be exacerbated. During routine clinical use these conditions can usually be identified easily before treatment. Thus, the side effects which are actually encountered are milder: the MRC trial (141) reported increased incidences of Raynaud's phenomenon, dyspnoea, rash, lethargy, nausea, dizziness and headache after propranolol. Reversible impotence was also noted, though the incidence was about half of that seen after bendrofluazide (i.e. 13% after direct questioning). The effects of the β-blockers on lipid metabolism remain the subject of controversy. Certainly, adverse effects on high density lipoproteins and triglycerides have been reported with several of these drugs (179).

Counterbalancing the potential harmful effects of long term β-blockade is the firm evidence that these drugs do have a secondary preventive action following myocardial infarction (93,154). It is pure supposition, but very tempting, to infer that they may be equally useful as primary preventive agents. The usefulness of the drugs as antianginal agents is beyond doubt.

Conclusions

This group of drugs having disparate properties and a common effect has now become widely used for cardiovascular indications. Contraindications are generally easily recognised and side effects are otherwise fairly minor though not infrequent. The potential cardioprotective effects have led to enthusiasm for choosing a β-blocker as first line therapy in hypertension. Nevertheless, there remains a cohort of patients who are unsuited to these drugs or in

whom a second drug is required.

Diuretics

General

The diuretics are drugs which share the property of promoting renal excretion of salts, and perhaps fortuitously, the property of lowering arterial blood pressure. There is wide variation in the potency and other pharmacological features of the diuretics. The most useful classification relies on their respective sites of action in the kidney. Thus, there are three groups in common use: the thiazides, which inhibit sodium reabsorption at the distal convoluted tubule (115); the loop diuretics, so called because they reduce sodium reabsorption in the ascending limb of the loop of Henle; and the potassium sparing diuretics, which are physiological antagonists (e.g. amiloride) or pharmacological antagonists (e.g. spironolactone) of aldosterone and thus prevent the exchange of potassium for sodium in the distal tubule and collecting duct (83). The diuretics which are most widely used in hypertension are the thiazides. Nearly 30 years of use illustrate their importance in this field.

Mode of Action

Clearly, prolonged diuretic exposure results in a resetting of the renal threshold for salt and water retention and in a reduction of intravascular and probably intracellular volume. This certainly contributes to the hypotensive action, but does not entirely explain it, since restitution of plasma volume will not completely restore the blood pressure (40) and since the dose response relationships

for diuresis and antihypertensive effect are not comparable (28). A combination of effects on vascular smooth muscle contractility (199), structural changes in resistance vessels due to alterations in intracellular salt and water (199) and the changes in extracellular volume is probably responsible for the antihypertensive effect.

Efficacy

The effect of the drugs is moderate, with a mean fall of about 12/8 mmHg (145). There does not appear to be any risk of precipitating sudden hypotension. In comparison with a β-blocker, there is little difference in efficacy (142). It is clear that a diuretic will potentiate the antihypertensive action of most other groups of drugs, and this is one of the most useful features. The thiazides are claimed to be more effective than loop diuretics in lowering blood pressure (6), but are probably equipotent to the potassium sparing agents (221).

Clinical Features

The MRC trial in mild to moderate hypertension illustrates the clinical features of bendrofluazide (141). Both sexes had significantly increased incidences of impaired glucose tolerance, lethargy, constipation and other reactions such as nausea, dizziness and headache. Men had strikingly higher incidences of gout and of impotence. With the exception of the hyperuricaemia, the side effects occurred especially within the first three months of exposure and usually within one year. Seventeen percent of men withdrew from bendrofluazide treatment over 5 years, compared with 13% of the women. Significant rises in serum uric acid and significant falls in serum potassium were noted. A marginal increase in serum cholesterol

occurred.

In contrast to the thiazide diuretics, the potassium sparing agents, amiloride and triamterine, have as their main side effect hyperkalaemia. Neither serum urate levels nor glucose metabolism is affected. Serum calcium, which is frequently raised by thiazides, is not affected by these drugs.

Spironolactone, in addition to producing hyperkalaemia, may cause gynaecomastia and impotence in men and menstrual disturbances in women due to its structural similarity to progesterone.

Conclusions

Diuretics, and particularly the thiazides, have an important role in hypertension management. The relatively high incidence of side effects in men and of biochemical changes which may increase long term risk does argue against first line use in all patients. For the patient in whom a β-blocker is contraindicated or inadequate, however, a diuretic is perhaps still the optimum second drug. The potassium sparing diuretics should be reserved for individual indications and the loop diuretics should only be used where fluid retention or renal impairment is present.

Vasodilators

These will be discussed under three headings: the direct vasodilators, the α-blockers and the calcium antagonists.

Direct Vasodilators

Hydralazine, minoxidil and diazoxide are the most commonly used members of this group. Hydralazine was first introduced in the 1950's but has only recently gained full recognition for its place in the modern regimen (137). The reasons for this are discussed below. Minoxidil is a more recent and very potent vasodilator. Once again, side effects limit its use. Diazoxide similarly is a potent vasodilator, but has adverse effects when used orally.

Mode of Action

These drugs all have a direct relaxant effect on vascular smooth muscle, which does not appear to be mediated by effects on adrenergic or other specific receptors. An effect on calcium channels has been reported with hydralazine (140) but as yet it is not recognised that this is the primary mechanism for its effect. The result of vasodilatation of the pre-capillary arterioles is a fall in vascular resistance. This is quite dramatic, but is compensated for by an increase in sympathetic activity which increases cardiac output (62). In addition, marked stimulation of the renin-angiotensin system occurs leading to fluid retention especially with minoxidil (165).

Efficacy

The vasodilating effect of these drugs is substantial, but the compensation which occurs limits their antihypertensive action. Combination with a β-blocker and diuretic greatly improves the results (137) although some workers would suggest that the β-blocker is superfluous in groups with poor baroreflex function, such as the elderly (5).

Clinical Features

The side effects of these drugs fall in to two categories. Those which are an extension of their pharmacological effect include tachycardia and fluid retention. These are not a major problem when the vasodilators are used in combination with the two usual first line drugs, a β-blocker and a diuretic.

Hydralazine has a further side effect, however. A small percentage of patients develop drug induced lupus with this drug. The risk is directly related to the dose, but although modern lower doses, under 150 mg per day, have led to a reduction in incidence, the syndrome continues to occur with an incidence of over 5% after one year's treatment (169). Slow acetylator status results in reduced drug clearance and predisposes to increased antihypertensive efficacy and increased risk of lupus in patients with this phenotype (164). Fortunately, withdrawal of the drug or even dosage reduction results in resolution of the syndrome, and regular monitoring of anti-nuclear factor titres may identify the at-risk patient before symptoms develop.

The unusual side effects associated with minoxidil include hirsutism

(57) and, perhaps simply as an extension of the fluid retention, pericardial effusion (135). Minoxidil is recommended only for hypertension in men, which is resistant to standard regimes.

Diazoxide is not used orally as it may cause significant glucose intolerance and renal impairment (162); it remains useful when given intravenously in hypertensive emergencies (147).

α-blockers

Three α-blockers are used for hypertension: prazosin, phentolamine and phenoxybenzamine. The last of these binds covalently to α-receptors and is almost exclusively used for pre-operative treatment of phaeochromocytoma. Likewise, phentolamine is not widely used, although it provides rapid and reversible α-blockade when given intravenously. Prazosin is therefore the only widely prescribed drug of this class.

Mode of Action

Prazosin is a specific antagonist at the post-synaptic α_1-receptor. Thus, it inhibits sympathetically mediated vasoconstriction and lowers total peripheral resistance.

Clinical Features

The first dose of prazosin occasionally causes profound hypotension, an effect which is minimised if a low starting dose is prescribed (78). After hydralazine, it was the most acceptable 'third drug' in the Glasgow 'Third Drug' Trial (137).

Calcium Antagonists

Two classes of drug are found in this group: the pyridine derivatives, typified by nifedipine, and the papaverine derivatives, such as verapamil. They are among the most recent and most promising of the antihypertensive agents to become available.

Mode of Action

Smooth muscle contraction is initiated by membrane depolarisation, which is sodium dependent, followed by calcium influx through specific channels; the calcium antagonists restrict this influx. Thus, they cause relaxation of vascular smooth muscle. There is some variation in the sites of action: verapamil also inhibits atrio-ventricular conduction and the smooth muscle of the gut, whereas nifedipine appears more specific to vascular muscle.

Efficacy

In the usual therapeutic doses, the calcium antagonists exert moderate antihypertensive effects, similar to β-blockers (64).

Clinical Features

Nifedipine has a similar effect to hydralazine on the peripheral vasculature and also causes mild compensatory tachycardia and oedema of the extremities. It does have the advantage, however, that coronary vasodilatation may occur and for this reason it is often used in the management of patients who have coexisting hypertension and angina pectoris. Pounding headache and a flushing sensation are common symptoms and the duration of action is short even when a sustained release formulation is employed.

Verapamil has the disadvantages of causing constipation (123) and of undergoing extensive first pass metabolism in the liver, thus making dosage selection difficult and variable. Its effects on the myocardial conducting system confer the advantage over nifedipine that a reflex tachycardia does not occur, but toxicity may result in heart block, especially in the presence of β-adrenergic blockade. Verapamil possesses useful antianginal activity (64).

Conclusions

In a condition characterised by increased peripheral resistance, such as hypertension, it would seem logical to employ vasodilator drugs. Homeostatic mechanisms operate which counter the fall in resistance, however, and which limit the effects on blood pressure and give rise to undesirable side effects. Future developments of these drugs may yet produce a compound which exhibits only the positive features of a compromise.

Centrally Acting Drugs

General

Reserpine, clonidine and α-methyl dopa comprise this group. The first of these is undoubtedly an effective antihypertensive agent (206,207) but it causes marked sedation or depression, sufficient even to result in suicide, and is not used in Britain at present. Clonidine has also lost favour, primarily because it has a short duration of action and the propensity to cause a hypertensive crisis after sudden withdrawal. Alpha-methyl dopa has been in use for a

quarter of a century, however, and although it is gradually being supplanted, it remains a widely prescribed drug.

Mode of Action

After crossing the blood brain barrier, α-methyl dopa is decarboxylated to α-methyl noradrenaline, which acts as a false transmitter (90). This compound is less potent than noradrenaline and may also inhibit the release of further noradrenaline by a presynaptic agonist effect. Thus, central maintenance of blood pressure is impaired and the haemodynamic result is a fall in both peripheral vascular resistance and cardiac output (176).

Clinical Features

In common with other centrally acting drugs, α-methyl dopa causes sedation. Peripheral effects include hypotension and occasional haematological or hepatic disturbances due to autoimmune mechanisms. A positive Coombs test is common but not a reason to discontinue treatment.

Conclusions

Despite the side effects seen with α-methyl dopa, it is a useful adjunct to the other drug groups and still is the mainstay of treatment of hypertension in pregnancy.

Angiotensin Converting Enzyme Inhibitors

This is an important new group of drugs with a novel mechanism of action. As the subject of this thesis, perindopril, is one of these drugs, the clinical pharmacology of the group will be discussed in greater detail in the next section.

General Conclusions Regarding Antihypertensive Treatment

From the foregoing discussion of the treatment of hypertension, two main conclusions can be drawn. The first concerns the decision on whether or not to treat certain groups of patients with drugs.

It is clear that the risk of stroke and cardiovascular disease extends down to 'mild' hypertensives, who have a diastolic blood pressure of over 90 mmHg. It is also clear that if these patients are treated, then only a very small proportion of them will accrue any benefit; yet a sizeable percentage of them will suffer side effects. Most of these side effects are temporary and will resolve on withdrawal of the drug. Many cause symptoms and will ensure that treatment will continue only when the treatment is well tolerated. Some drugs, however, carry the risk of biochemical toxicity which may go unrecognised or ignored. Although the major hypertension trials have reported follow-up periods of several years, there is no satisfactory evidence about the risk to benefit ratio of very long term treatment and yet this is the logical result of a decision to treat young or middle aged mild hypertensive patients. The clinician must balance in his mind the relative weights of evidence. The crux

of this point is that the safer and more acceptable is the available treatment, the easier is the decision to exhibit that treatment.

The second conclusion concerns the currently available antihypertensive drugs. None is free from side effects and Ehrlich's concept of a 'magic bullet' remains an abstract dream. Present treatment is, nevertheless, vastly better than the drugs available at the time of the first trial of antihypertensive therapy. The preceding discussion of the groups of drugs highlights the differing features of each group but it also emphasises that within the groups are drugs which have disparate characteristics. It is reasonable to conclude that novel compounds will be discovered in the future which will further augment the armoury of the physician. It is also reasonable that research on new members of the recognised groups of antihypertensive agent should proceed. Each improvement in treatment which ensues is merely a small step but it does represent progress.

1.3 ANGIOTENSIN CONVERTING ENZYME INHIBITORS

A case has been argued for the development of new antihypertensive compounds. The investigation of such drugs is most likely to prove worthwhile where a novel mechanism of action is involved or where existing members of a drug group are few in number. This thesis concerns the early clinical development of one new compound in a relatively recent group of drugs, the angiotensin converting enzyme inhibitors. At present, only two compounds in this group are available for routine clinical use in the United Kingdom. Brief

mention has already been made of their novel mechanism of action. Understanding of the effects of these drugs requires some background information on the physiology of the renin-angiotensin system. This homeostatic system is therefore reviewed before the history and clinical pharmacology of the angiotensin converting enzyme inhibitors is described.

Physiology of the Renin-Angiotensin System

The hydrolysis of angiotensinogen to form angiotensin I is catalysed by an enzyme, renin, in the blood. This forms the first step in the release of angiotensin II from its precursor α_2-globulin. Thereafter, the carboxyl terminal dipeptide is rapidly cleaved by angiotensin converting enzyme to yield the octapeptide, angiotensin II.

Renin

Renin is an acid protease with a molecular weight in its active form of about 40,000. It is fairly species-specific. Although originally thought to be localised to the kidney and plasma, renin activity has now been demonstrated in many other organs including salivary glands (39), the reproductive system (192), brain (66), blood vessels and adrenals (174). The enzyme is stored in intracellular granules until release. In the kidney, the renin-rich cells are modified endothelial cells of the afferent glomerular arterioles, which lie in close approximation to the macula densa of the distal convoluted tubule and which together form the juxtaglomerular apparatus (77). This apparatus is sensitive to a reduction in the blood pressure and

salt concentration which it perceives. It responds by releasing renin in to the circulation (46). The enzyme which is released is not all active. Cold, trypsin and a variety of proteases are capable of activating renin (10,48,49,118). Specific inhibitors of renin have been elaborated (85) but none is yet available for clinical use.

Angiotensin

The substrate upon which renin acts is a circulating α_2-globulin which is synthesised by the liver. Although the naturally occurring form comprises a series of high molecular weight glycoproteins, it appears that only the terminal 14 amino acids are essential to act as a substrate for production of angiotensin I (183). Thus, synthetic substrates, both active and inhibitory, have been created (56). Angiotensin I is a decepeptide which probably has no biological activity. One major feature, however, is that it interferes with radioimmunoassays for angiotensin II and may cause falsely high results, particularly when the ratio of angiotensin I to angiotensin II is high.

Angiotensin II is the active octapeptide which remains after hydrolysis of the C-terminal dipeptide from angiotensin I. The majority of angiotensin II is formed by this route, but alternative pathways from angiotensinogen to angiotensin II have been shown to exist. One of these relies on the action of another enzyme, tonin, and probably obviates the need for angiotensin converting enzyme (19).

Angiotensin II has two principal effects in vivo. Firstly, it is a potent vasoconstrictor by a direct action on vascular smooth muscle,

by facilitating peripheral catecholamine release and possibly by central actions which increase sympathetic nervous activity. Secondly, angiotensin II causes salt and water retention by stimulating aldosterone release, by a direct effect on tubular sodium reabsorption and by central effects which include enhancement of thirst, salt appetite and release of antidiuretic hormone and adrenocorticotrophic hormone. Angiotensin II is inactivated by peptidases, firstly to angiotensin III and then to inactive fragments. The biological activity of angiotensin III remains in doubt (76).

Angiotensin Converting Enzyme

This is the enzyme responsible for the conversion of angiotensin I to angiotensin II. It is a glycoprotein with molecular weight of 130,000 - 160,000 (184). It is a zinc containing enzyme which is activated by chloride and inhibited by metal-chelating agents. It acts as an exopeptidase, releasing dipeptides from the carboxyl end of a variety of peptides, including angiotensin I, bradykinin, enkephalins and substance P (184). Thus, angiotensin converting enzyme is also described as kininase II, the main inactivating enzyme of bradykinin.

Angiotensin converting enzyme is found circulating in the blood, but it is mainly localised to the cell membrane on the luminal side of vascular endothelial cells, renal tubular cells and the epithelial microvilli of the gut (175). Thus, a significant proportion of its substrate is converted across the pulmonary circulation: some 80% of angiotensin I and 90% of bradykinin is dealt with in a single passage of the lungs (158). The lung only accounts for about 45% of

angiotensin I conversion to angiotensin II, however, since other tissues have access to angiotensin I before it reaches the lung (38). It is apparent that not only is angiotensin converting enzyme closely involved in the production of angiotensin II, it also is responsible for inactivating bradykinin. Some description of the kallikrein-kinin system is therefore relevant.

The protease enzyme, kallikrein, is found in a distribution analagous to that for renin. It catalyses conversion of a kininogen, which in contrast to angiotensinogen has low molecular weight, to lysyl-bradykinin. This hormone is inactivated by further peptide hydrolysis, angiotensin converting enzyme being one of the participating kininases. Bradykinin causes potent vasodilatation, natriuresis and diuresis. Its circulating levels are not consistently altered by inhibition of angiotensin converting enzyme and therefore it is not yet clear to what extent the kallikrein-kinin system contributes to the effects of the angiotensin converting enzyme inhibitors. Nevertheless, it is possible that local changes in bradykinin levels occur which are not reflected in circulating levels but which are sufficient to cause haemodynamic effects.

Manipulation of the renin-angiotensin system has fascinated hypertension workers and physiologists for many years. Goldblatt demonstrated in a series of elegant experiments the effects of renal ischaemia on the blood pressure (74) and inhibitors of renin are still being studied. The aldosterone antagonist, spironolactone, is useful in the treatment of hyperaldosteronism. The only drugs with more general applicability which specifically modulate the renin-angiotensin system and which are clinically available are the

inhibitors of angiotensin converting enzyme. The history and clinical pharmacology of this group will be reviewed in more detail.

Clinical Pharmacology of Captopril and Enalapril

History

When Ferreira identified a bradykinin potentiating factor derived from the venom of Bothrops jararaca in 1965 (58), it was still not recognised that angiotensin converting enzyme and kininase II were the same enzyme. In 1968, Bakhle confirmed that bradykinin potentiating factor also inhibited angiotensin converting enzyme (13). It was 1970 before Yang published work concluding that they were the same enzyme (224). Bradykinin potentiating factor was subsequently purified and identified as a series of peptides which could be synthesised (157).

Whilst one of these peptides, teprotide, was very effective as an antihypertensive agent (68), it suffered the disadvantages of its peptide structure: it was not orally active, it had a short duration of action and it was expensive to synthesise. Nevertheless, the structure activity relationships which were established from teprotide and its congeners together with detailed knowledge of the structure of angiotensin converting enzyme allowed synthesis of further series of compounds with suitable inhibitory effects (156).

Chemistry

The two compounds which have been marketed in Britain at this time show structural dissimilarity: captopril is a mercaptoacyl amino acid whereas enalapril is a carboxyalkyl dipeptide (Figure 1). Enalapril is the ester of the more active compound, enalaprilat.

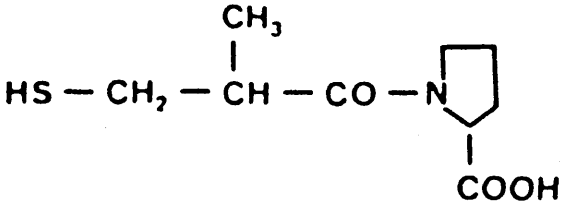
Mode of Action

Current knowledge of the physiology of the renin-angiotensin sytem and of the kallikrein-kinin system suggests two main mechanisms by which the angiotensin converting enzyme inhibitors may lower blood pressure. A reduction in circulating angiotensin levels may lead to loss of direct and indirect vasoconstriction. A rise in bradykinin levels due to diminished catabolism might cause vasodilatation.

No rise in systemic levels of bradykinin has been reported, but it has been postulated that local increases in levels of this hormone may still occur and may be responsible for a fall in total peripheral resistance (107). Animal work with anti-kinin antibody has demonstrated that the antibody can prevent the fall in blood pressure associated with captopril given to rats with renovascular hypertension but does not affect the hypotensive action of captopril given to salt-depleted animals (29). Captopril could not be demonstrated to enhance the effect of bradykinin infusion locally to the kidney (59).

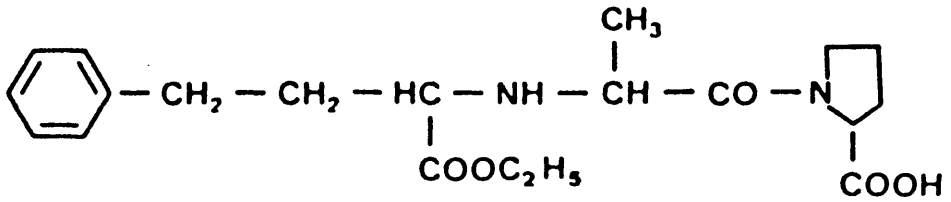
There is evidence of a relationship between bradykinin and prostaglandin E_2 release (148). Angiotensin converting enzyme inhibition does increase prostaglandin E_2 production in renomedullary interstitial cells (226). Captopril has been shown to

SQ 14,225 (captopril)



E.R. Squibb

MK421 (enalapril)



Merck Sharp and Dohme

Figure 1. Chemical structure of captopril and enalapril.

augment prostaglandin E_2 production in hypertensive man and indomethacin, which is an inhibitor of cyclo-oxygenase, can prevent the hypotensive action of captopril (191). Indomethacin also antagonises other antihypertensive drugs, however (55,173,212). Further, angiotensin II infusion has also been shown to augment prostaglandin E_2 release (138). Thus, the role of bradykinin in the mechanism of hypotension after angiotensin converting enzyme inhibitors is still a matter for debate.

As regards the renin-angiotensin system, investigators have variously reported that the antihypertensive effects of the angiotensin converting enzyme inhibitors are closely correlated (7,31,42), sometimes correlated (21) and not related (131) to the plasma renin activity before dosing. A degree of salt or volume depletion is necessary for the blood pressure lowering effect when plasma renin activity is not high, however (33,131). The angiotensin converting enzyme inhibitors do lower angiotensin II concentrations (24,100). Thus, a reduction in both direct and sympathetically mediated vasoconstriction occurs (37,198). The modulation of the sympathetic neural effects appears to occur even with low initial levels of angiotensin II (108), which helps to explain why anephric patients may still respond to angiotensin converting enzyme inhibitors (131). Angiotensin II levels may regulate the number of angiotensin II receptors (84) and, therefore, the effects of angiotensin converting enzyme inhibitors might reasonably be expected to occur, regardless of the plasma renin activity.

Since neither captopril (125) nor enalapril (96) consistently lowers aldosterone after long term treatment, it seems unlikely that effects

on aldosterone are responsible for the long term antihypertensive action.

Circulating catecholamines have been shown to fall (215), to be unchanged (136,153) and, in certain instances, to increase (146) after angiotensin converting enzyme inhibition. Since an enhanced response to catecholamines (91) and possibly increased resting levels (34) have been implicated in essential hypertension, an interaction between angiotensin converting enzyme inhibitors and the sympathetic nervous system via angiotensin II could conceivably explain the hypotensive action of these drugs.

One area of particular interest has been the effect of the angiotensin converting enzyme inhibitors on heart rate control. It is well established that vasodilatation occurs with these drugs, but that the reflex tachycardia which is seen with many vasodilators does not occur (61,102,143,193) though the response to tilt (14) and exercise (132) is unaffected. Clearly, this could be due to removal of angiotensin II mediated facilitatory effects on the sympathetic nervous system; yet the evidence suggests that parasympathetic rather than sympathetic mechanisms may be responsible for the tachycardia after other vasodilators (130). Saralasin, the angiotensin II partial agonist, reduces heart rate in dogs and this effect can be abolished with atropine (20). Captopril caused enhancement of bradycardia in the diving reflex test in hypertensive patients (188). Finally, central effects of angiotensin II on heart rate seem to be mediated by the vagus nerves (177). Interestingly, the central effects of angiotensin II on blood pressure are also abolished by atropine (177) and by ablation of the area postrema in

dogs (110).

Absorption, Metabolism, Excretion

Initial studies of the pharmacokinetics of captopril were confounded by the instability of the drug *in vitro* (172). Published reports must therefore be treated with caution. Studies using radiolabelled compound have yielded some information, however.

Captopril is rapidly absorbed after oral administration, reaching peak blood levels in one hour, and has oral bioavailability of 62%. The volume of distribution at steady state is 0.7 $l.kg^{-1}$ and the elimination half life is 1.7 - 1.9 hours (53). Three-quarters of an oral dose is excreted in the urine, 58% in an unchanged form, 40% as polar metabolites and 2% as disulphide (114). Elimination is reduced in renal failure (171) and by coadministration of probenecid. Tubular secretion is thus important (182).

Enalaprilat is poorly absorbed, but absorption of its esterified form, enalapril, exceeds 60% and over 70% of enalapril which is absorbed is hydrolysed to the active metabolite (203), probably in the liver (200). Peak blood levels of enalaprilat are achieved within four hours of administration (17,143). Urinary recovery of enalapril and enalaprilat together accounts for 61% of the administered dose, and faecal recovery offers a further 33% (203). Reduced clearance in the elderly has been shown to correlate with diminished creatinine clearance (94).

The kinetic profile of enalaprilat is polyphasic, with a long terminal phase (94,203) but, despite an apparent terminal

half-life of 35 - 38 hours (94,203), Till et al (197) estimate the accumulation half-life to be only 11 hours. This has led to controversy over the relevance of standard pharmacokinetic models to the description of the disposition of enalaprilat. It has been suggested that protein binding of enalaprilat occurs and that, as a result, a small pool of drug is excreted very slowly.

Efficacy

Both captopril and enalapril are of proven benefit in several forms of hypertension and in congestive heart failure. They may be used in renovascular hypertension (25,95,96) and may predict the response to surgery (9). Caution is necessary, however, since renal function may deteriorate if the only functioning renal tissue is distal to a stenosis (99). Fortunately, the change in renal function is usually reversible. It has also been suggested that captopril may be a diagnostic aid in renal artery stenosis: it causes a greater rise of plasma renin activity in renal artery stenosis than in essential hypertension (30) and causes exaggeration of the asymmetry seen in functional renal isotope scans when unilateral renal artery stenosis is present (216).

These drugs have been used successfully in the treatment of severe and malignant hypertension (32,51). They are effective in primary hyperaldosteronism without tumour (82,133), possibly because this condition is due to increased adrenal sensitivity to angiotensin II (222).

Of greater importance numerically are the patients with essential hypertension. Many reports are now available showing benefit with

either captopril or enalapril in these patients (67,208,209). These studies have used twice daily administration of captopril, but comparison of once daily and twice daily administration with enalapril has shown equal benefit with the single daily dose (16). Coadministration of a diuretic is often required to achieve an optimal effect in patients with essential hypertension.

The value of angiotensin converting enzyme inhibitors in congestive heart failure is now fairly well recognised (36,202). Cardiac output rises while blood pressure, total peripheral resistance, right atrial pressure, pulmonary artery pressure and pulmonary wedge pressure are lowered (112,122). The fall in blood pressure may sometimes be profound, but it has been suggested that this is usually well tolerated because of a simultaneous increase in cerebral blood flow (105). The mechanism of the falls in afterload and preload are not clear: removal of a direct action of angiotensin II is unlikely, since angiotensin II does not directly affect the venous system (159) and since rebound rises in angiotensin II levels after withdrawal of captopril do not cause arterial vasoconstriction (152). Significant subjective and objective improvements in exercise tolerance have been reported after angiotensin converting enzyme inhibitors and there has been a suggestion that survival is improved (113). A reduction in complex ventricular arrhythmias has been noted (36).

Clinical Features

The reported side effects with captopril must be assessed with care. The initial studies with captopril in patients concentrated on the treatment of unusual or high risk disease. Full clinical pharmacology was not known at the time and certain errors were made

in dosing which were avoided when enalapril became available. Thus, a comparison of the incidence of side effects with the two drugs must allow for major differences in the populations at risk.

The side effects commonly encountered with the angiotensin converting enzyme inhibitors are rash, taste disturbance, headache, lassitude, hypotension, proteinuria and neutropenia. The respective incidences of these complications for captopril are 6%, 3.1%, 2.9%, 2.7%, 2.5%, 0.6%, and 0.04% and for enalapril are 1.5%, 0.5%, 5.6%, 5.1%, 2.4%, 1.4% and 0.06% (187). The at-risk group for captopril in the above report comprised nearly 5000 patients of whom over 90% had moderate to severe or renovascular forms of hypertension, whereas the 2000 patients given enalapril included only 18% such 'high risk' patients. That this is relevant is evidenced by the strikingly higher incidence of haematological side-effects with captopril in patients with collagen disease and renal failure together (7.2%) compared with patients having renal failure alone (0.4%) or normal renal function (0.02%). Since captopril is renally excreted (89) and most of the side effects are now known to be dose-related (208) it may be that the differences between the two drugs are not so great as once was thought. This point is disputed by the manufacturers of enalapril, however (75,144).

Both rash and taste disturbance are usually dose-related. Cough and angioneurotic oedema may occur with either drug (106). Proteinuria occurs with both drugs in similar numbers of patients. The nephropathy was originally attributed to the sulphydryl group in captopril, and some supporting biochemical work was published (103). The similar incidence with enalapril and the relatively low incidence

with both drugs in contrast to the incidence seen with penicillamine, which causes proteinuria in 20% of patients treated, argue against this mechanism and have led to greater tolerance of captopril by physicians in recent years.

On the positive side, the combination of an angiotensin converting enzyme inhibitor with a thiazide diuretic has been reported to reduce the incidence of some of the biochemical side effects of the diuretic (214). An additional feature of the angiotensin converting enzyme inhibitors is that they lower blood pressure without causing reflex sympathetic activation (44) and are, therefore, particularly useful in the treatment of hypertension complicating angina, although a β-blocker would normally remain first line treatment.

Finally, there have inevitably been some reports of unusual illnesses during captopril treatment (8,163). These cannot be confidently attributed to the drug at present.

1.5 INITIAL INVESTIGATION OF NEW DRUGS IN MAN

The rationale for investigating a new antihypertensive agent has been set out above. When the studies of the clinical pharmacology of perindopril (Chapters 3 to 8) were planned, limited information was available concerning administration of the drug to man. It is therefore relevant to disuss the procedures involved in the initial stages of the clinical investigation of a new drug.

Introduction

The development of a new drug follows a fairly standard path. After synthesis or discovery of the new compound, it is tested *in vitro* and in animal experiments for particular pharmacological properties. If it possesses useful activity, it is then subjected to rigorous examination of its pharmacokinetic properties and toxicology in animals. Only if it continues to show promise does it continue beyond this preclinical stage.

The clinical stages have been classified into four phases: phase 1 covers the first administration to man, usually healthy volunteers, and assessment of disposition and tolerance; phase 2 extends the earlier work to establish early evidence of efficacy, appropriate dosage and further assessment of safety; phase 3 permits the detailed examination of efficacy and comparison with any established treatment, along with further assessment of safety; phase 4 covers

the period after marketing when efficacy has been accepted but surveillance for safety in large numbers of patients is necessary.

In contrast to the extensive literature on the subject of controlled clinical trials of drugs during phase 3 of their development, there is little on the subject of initial studies of a new compound. Several points may be considered.

Firstly, whilst it has been advocated that administration to humans should be a very early step in the development of a new drug (189), albeit under strictly controlled conditions, there is general agreement that adequate pharmacological and toxicological data should first be obtained in animals (22,52,70,73,79,117,204). The second point is that no strict criteria may be laid down for the conduct of such studies: the potential toxicity or efficacy of the compound dictates the manner in which these are approached. Thirdly, as a corollary to the latter point, the aims of such studies vary between compounds. Fourthly, some discussion should be devoted as to who should conduct early studies in man. Finally, certain ethical and legal considerations pertain to the conduct of these investigations. These points will be dealt with in the following paragraphs.

Legal Requirements

The restrictions upon administration of novel compounds to man and the requirements for documenting the early studies to allow for future licensing of the drugs vary from one country to another. For example, France does not in theory permit the conduct of studies in normal volunteers, the USA has a long tradition of recruiting volunteers from prison inmates and Britain has at present no

restriction on administration of unlicenced compounds to normal volunteers, yet it does exert strict control over administration to patients. Likewise, the American Food and Drug Administration differs in its requirements before licensing from the United Kingdom's licensing authority. The regulations discussed hereafter are restricted to those of Great Britain and Northern Ireland.

In the decade from 1971 to 1981, there was a deal of discussion and criticism of the onerous requirements to be met before a drug could be tested in patients (22,41,69,70). There was evidence that British companies were sending their new discoveries abroad to be tested and that clinical pharmacologists in this country were not gaining necessary experience in the early clinical trials (80). Part of the blame for this was attributed to the delay which occurred, after pre-clinical work had been completed, before a clinical trial certificate was granted. Thus, in 1981, new regulations came in to force (98).

The essence of these regulations was that they expanded a loophole in the Medicines Act 1968. Following an amendment to that Act in 1972, it had been possible for a clinician acting on his own initiative to apply for exemption from holding a clinical trials certificate for a particular compound (97). This was ordinarily granted after only 'negative vetting' of the information about the drug under consideration. This contrasts with the 'positive vetting' of the detailed pharmacological and toxicological data demanded of the pharmaceutical industry before granting a Clinical Trials Certificate. The detailed vetting is normally carried out on behalf of the licensing authority (i.e. the Secretary of State attached to

the Department of Health and Social Security, through the Medicines Division of that Department) by the Committee on Safety of Medicines.

Thus, since 1981, the pharmaceutical companies have still been required to generate the same basic data to support the proposed study. They have been required to send to the licensing authority only certified summaries of these and other relevant data, however, together with a copy of the protocol and a supporting letter from a medical adviser to the company, working in the United Kingdom.

The licensing authority has 35 days in which to consider the application, with an option of a 28 day extension. If granted, the onus is placed upon the exemption holder to report any change in the protocol, any adverse reaction to the drug, any other information casting doubt on safety and any refusal by an ethical committee to approve the trial.

Griffin has clarified the position of ethical committees in this respect (81). There is no extra responsibility in the case of Exempted Trials: they continue to consider the ethics of the trial as a whole.

The reported increase in the number of early clinical trials being conducted under the revised procedures and the absence of evidence suggesting any change in the adverse event rate attest to the value of the revised procedures (185).

Ethical Considerations

A full discussion of the ethical considerations involved in the early clinical trials of a new drug is outwith the scope of this review. Certain guidelines have evolved, however, which are compatible with the Declaration of Helsinki. The project and protocols must be approved by a local ethical committee. Informed consent must be voluntarily given, in writing, and independently witnessed. Written information on the purpose of the study, the procedures, risks and discomfort anticipated should be provided. It must be clear to the volunteer or patient that he may withdraw from the study at any stage and without prejudice to his medical care.

Subjects

The choice of subjects for phase 1 and 2 studies depends on several factors. The nature of the drug to be tested is the first of these. There is agreement that anti-cancer drugs, because of their low therapeutic index, should be tested only in patients with that disease (52,127) and that certain compounds can only be tested for an effect in patients with the relevant condition. Dollery cites as an example the use of L-dopa for Parkinsonism (52). For drugs with a safety profile which is thought to be good and in studies where assessments of safety, tolerance and clinical pharmacology rather than of therapeutic efficacy are the aims, there are good arguments for recruiting normal volunteers. These subjects provide a stable milieu in which detected changes are likely to be drug induced. There will be less variation in response and therefore more confidence in the results. A caveat to the sole use of 'normal healthy volunteers' is that further studies may be required to show the relevance of early data to heterogeneous groups, such as the

elderly (205).

The source of normal volunteers has vexed some minds in the past (12) as have the criteria for their selection (109). A good case exists for restricting recruitment from the staff of a company which will subsequently market a drug, on the grounds that undue pressure on the 'volunteer' cannot be excluded. Prison inmates may be similarly placed, though Ayd argues otherwise (12). Recruitment amongst staff in an independent department of clinical pharmacology probably avoids the problem of undue pressure and also ensures some prior knowledge of risks and procedures involved. Laurence writes that he favours the situation where investigators frequently 'renew the experience of being an experimental subject' (117).

Investigator

This leads to the choice of clinical investigator. The views of clinical pharmacologists are in accord on this subject: an independent clinical pharmacologist with appropriate training and experience, working in a well equipped and staffed laboratory within a hospital should be responsible for the first administration of a new compound to man (52,73,117,204). Subsequent trials of efficacy in patients may be carried out by clinical specialists. There is of course scope for flexibility between these stages.

Aims of Early Studies

George (69) has described the purpose of clinical studies as threefold: 'firstly, to establish that the drug has a useful action in man; secondly, to confirm that it is non-toxic; and, thirdly, to establish the nature of common side effects'. In practical terms,

however, early studies cannot answer these questions adequately. For example, serious toxic effects may have an incidence of one in 10,000 or lower and so will be unlikely to occur during early studies (23). The aims of phase 1 studies have therefore been simplified to assessment of the following (204):

1) human tolerability of the compound
2) pharmacokinetic behaviour and metabolism of the new investigational drug
3) effects of the compound on physiological functions
4) preferred route of administration
5) safe dosage range

The aims of phase 2 studies will also attempt to consider:

1) efficacy
2) optimal dosage range
3) side-effects and toxicity
4) choice of pharmaceutical formulation
5) mechanism of action

Inevitably, it is not possible to assess more than one or two points in a single study. Not all of these will be relevant for all new drugs, however: route of administration and formulation may have been decided beforehand; mechanism of action may be clear from pre-clinical pharmacology. Since it is clearly inappropriate to assess tolerability at a subtherapeutic dose, and since neither therapeutic dose nor safe dosage range have been established until phase 1 studies have been completed, the first practical step must be to perform a dose-ranging study.

Goldberg et al (73) suggest that the starting dose should be

considerably below the minimum effective dose in animals and that logarithmic increments in dose are sensible; they freely admit, however, that the choice of dose and of increment is arbitrary. They recommend a minimum of three subjects be given each dose. Various authors reach a firm consensus on one point: no firm guidelines can be laid down for a standard phase 1 study of any drug (52,69,73,117, 204). Each compound must be considered on its merits and in the light of the available pre-clinical data, likely safety profile, etc.

Finally, phase 3 studies should aim to demonstrate efficacy and superiority over existing treatments: the comparison should include safety, efficacy, acceptability and probably cost. Proof of equality is probably insufficient to justify marketing though this is not yet a widely accepted point.

Pre-clinical data

No attempt will be made to list the necessary pre-clinical data on a new drug, nor to review the methods of obtaining or assessing such data. In general terms, however, the information should cover three topics (117,204):

1) the action, efficacy and mechanism of the drug - the pharmacodynamics
2) absorption, metabolism and excretion - the pharmacokinetics
3) animal toxicology

Conclusions

From the preceding discussion, it should be clear that the development plan for a new drug through the early clinical stages is heavily dependent on the preclinical data for that compound and on

any available information concerning the effects of similar drugs in man. The aims of the early studies have been identified. An outline of the regulations and the consensus view on conduct of such investigations has been presented.

1.5 SCOPE OF THESIS

The study of the clinical pharmacology of the drug which forms the subject of this thesis, perindopril, offers a specific example of the application of many of the above points. The preclinical data for perindopril are summarised in Appendix 1. Some of the animal pharmacology has now been published (50,116). Limited information was available, in the form of unpublished work from the manufacturers (Institut de Recherches Internationales Servier), who had administered single doses of perindopril orally to pairs of volunteers up to 16 mg without obvious side effects or toxicity.

The studies on perindopril which follow were designed in the light of the safety profile in animals and of the knowledge which is now available regarding captopril, a drug with a similar pharmacological profile to perindopril, but more particularly based on experience with enalapril, a drug with similar pharmacology and structure to perindopril.

It was necessary not only to have means of measuring drug action (blood pressure, heart rate, plasma angiotensin converting enzyme activity and hormones) but also to develop a new assay for the metabolite of perindopril in plasma (Chapter 2).

The first series of studies, involving some of the earliest administrations of perindopril to man, established the dose-response relationship of single and multiple doses in normal subjects (Chapter 3). The first ever administration to man of the active metabolite, S-9780, is described in Chapter 4. Chapter 5 covers the interaction of perindopril with the autonomic nervous system in normal subjects. Early phase 2 studies in essential hypertension permitted the evaluation of the dose range predicted from the earlier studies and confirmed antihypertensive efficacy and good tolerance (Chapter 6).

Finally, in Chapters 7 and 8, the pharmacokinetics of the drug and the concentration-effect relationships are examined using a novel approach to permit an integrated overview of the actions of this new drug in man.

CHAPTER 2

LABORATORY METHODS

2.1 PLASMA ANGIOTENSIN CONVERTING ENZYME ACTIVITY

Plasma angiotensin converting enzyme activity was assayed by measuring the rate of generation of hippuric acid in an incubation mixture containing plasma and hippuryl-histidyl-leucine (43). Hippuric acid was measured by high pressure liquid chromatography as described by Chiknas (35).

Reagents and Materials

Hippuryl-L-histidyl-L-leucine, hippuric acid and phthalic acid were obtained from Sigma Chemical Company Limited, Dorset. All other reagents were 'A.R.' grade as supplied by Fisons Limited, England.

Chromatography

A Hewlett Packard 1084B with a Hewlett Packard 79850B LC terminal was used, with a Hichrom Spherisorb 5 μm ODS column (12.5 cm x 4 mm ID). The detector was a Pye Unicam LC3 UV detector set at 228 nm. The mobile phase used was 20 mM KH_2PO_4, pH 4.6 : methanol 40 $ml.l^{-1}$ at a flow rate of 2 $ml.min^{-1}$.

Sample Requirements

At least 100 μl of plasma or serum are required for angiotensin converting enzyme analysis. It has been shown that there is no significant difference between plasma and serum angiotensin

converting enzyme activity. Samples collected in EDTA are unsuitable for angiotensin converting enzyme analysis as EDTA has been found almost completely to inhibit the enzyme.

Samples from patients taking no angiotensin converting enzyme inhibitor can be stored for up to 6 months at -20^{o}C without any appreciable decrease in enzyme activity.

It was found that the angiotensin converting enzyme activity of haemolysed samples was decreased by up to 50%.

Methodology and Standards

Standard Solutions

A standard curve for hippuric acid was constructed by spiking the plasma of a normal volunteer with varying amounts of hippuric acid (0.05 - 0.5 mmol.l^{-1}). These standards could be kept at -20^{o}C for at least 2 months, with frequent defrosting and refreezing having no effect.

Internal Standard (IS)

Phthalic acid for use as an internal standard was used at a concentration of 200 μM. This could be stored at 4^{o}C for at least 2 months without significant degradation occurring.

Substrate

Five mmol of hippuryl-L-histidyl-L-leucine were dissolved in substrate buffer. This was made up freshly for each assay as there is a gradual increase in free hippuric acid with storage. The substrate buffer was 100 mM potassium phosphate buffer pH 8.3 containing

300 mmol NaCl per litre.

Method

Standards and unknown samples were assayed slightly differently:

Plasma standards in duplicate

400 µl substrate buffer containing no Hip.His.Leu

+ 40 µl 50% HCl	vortex
+ 20 µl standard plasma	vortex
+ 40 µl IS	vortex
+ 10 mg (approx) NaCl	vortex
+ 800 µl ethyl acetate	vortex for 15 seconds

Unknown samples in duplicate

400 µl substrate buffer containing Hip.His.Leu

+ 20 µl unknown plasma	vortex
	incubate at 37°C for 15 minutes
+ 40 µl 50% HCl	vortex
+ 40 µl IS	vortex
+ 10 mg (approx) NaCl	vortex
+ 800 µl ethyl acetate	vortex for 15 seconds

Plasma standards and unknown samples, continue as follows:

Centrifuge for 5 minutes at 2000 rpm

Remove 400 μl organic layer to clean tube

Concentrate under N_2/air at 37^{o}C

Reconstitute in 100 μl mobile phase

Inject aliquot into HPLC system

Calculation of Angiotensin Converting Enzyme Activity

Results were calculated using peak area. The peak area ratio for hippuric acid/IS was plotted against hippuric acid concentration. The hippuric acid/IS ratio for the unknown sample was then read off the graph, giving a value in mmol of hippuric acid per litre generated in 15 minutes. One unit of angiotensin converting enzyme activity is that which converts 1 μmol of angiotensin I to angiotensin II per minute.

Sensitivity and Reproducibility

Inter and intra assay coefficients of variation were 6.1% and 2.3% respectively and the limit of detection was 0.5 $EU.l^{-1}$. Samples were assayed within four weeks of collection.

Normal range

The normal range for the laboratory was 15.3 - 26.9 $EU.ml^{-1}$ (95% confidence interval).

2.2 PLASMA RENIN ACTIVITY

Plasma renin activity was determined by a specific radioimmunoassay of angiotensin I formed upon cleavage by renin of its substrate angiotensinogen (49). The limit of detection of the assay was 1 $ngAI.ml^{-1}.hr^{-1}$ and the inter and intra assay variations were 7.0% and 5.5% respectively.

2.3 PLASMA ALDOSTERONE

Plasma aldosterone concentrations were measured by a direct radioimmunoassay using the method of McKenzie et al (139). The limit of the assay was 10 $pg.ml^{-1}$ and the inter and intra assay coefficients of variation were 11.0% and 7.3% respectively.

2.4 PLASMA CATECHOLAMINES

Adrenaline and noradrenaline concentrations in plasma were determined by a radioenzymatic assay based on the method of da Prada and Zurcher (167). The method is dependent upon the determination of the methylation of the catecholamines with tritiated s-adenosyl methionine by the action of purified catechol-o-methyl transferase. The limit of detection for noradrenaline was 0.1 $nmol.l^{-1}$ and for adrenaline was 0.03 $nmol.l^{-1}$. The inter and intra assay variations were respectively 15% and 13% for noradrenaline and 20% and 15% for adrenaline.

2.5 PLASMA S-9780 CONCENTRATIONS

Plasma S-9780 concentrations in plasma were estimated by an enzyme-inhibition assay based on the method of Tocco et al (200). The assay was developed in the laboratories of the Department of Materia Medica. The diacid metabolite of perindopril, S-9780, was separated from plasma using an ion exchange column. Subsequent analysis of metabolite concentration depended upon the degree of inhibition of a standardised angiotensin converting enzyme preparation using high pressure liquid chromatography to detect the product of the reaction.

Methodology and standards

Five hundred μl of plasma was adjusted to pH 2 by the addition of 50 μl of 0.1 M hydrochloric acid. This was then poured on to an XAD-4 column and the tube was rinsed twice with 2 ml of 0.1 M HCl, the rinses being added to the column. The column was then washed with a further 6 ml of 0.1 M HCl added slowly in 3 aliquots. The sample was eluted from the column with 3 x 1 ml methanol and the methanol was then reduced to dryness under a stream of nitrogen at 45^{o}C. The samples were reconstituted with 0.2 ml methanol and 0.8 ml of angiotensin converting enzyme buffer (phosphate buffer 0.5 M, pH 8.3, and 0.6 M NaCl).

The HPLC assay of Chiknas et al (35) was then employed to determine the inhibition of a standardised preparation of angiotensin converting enzyme. One hundred μl of reconstituted eluant were added

to 400 μl of substrate (hippuryl-histidyl-leucine). Substrate concentration in the assay system (5 mM) was such that more than 80% remained unchanged after the incubation with the enzyme. Fifty μl of diluted rabbit plasma were added such as to give a final dilution of 1 in 33 of the rabbit plasma in the enzyme assay system.

Samples were then incubated for 15 minutes at 37^{o}C, the reaction being stopped with 0.1 ml of 6 M HCl. Internal standard was added (15 μl of 0.1 mM o-phthalic acid), followed by approximately 10 mg of NaCl and 0.8 ml of ethyl acetate. After mixing, the samples were centrifuged and 0.5 ml of supernatant were evaporated to dryness under nitrogen at 60^{o}C. This was then reconstituted with 0.1 ml of HPLC mobile phase (60% 0.02 M phosphate buffer pH 8.0 and 40% methanol) and 25 μl of this was injected into the chromatograph. Separation was effected on a 25 cm 5 μm ODS spherisorb column at a flow rate of 2 ml.min^{-1}.

Hippuric acid generated by the action of rabbit plasma angiotensin converting enzyme was detected at 228 nM and was related to the internal standard by peak height ratios. Each batch of samples was assayed along with a series of standard callibrators (0 - 25 ng.ml^{-1}) along with two quality control standards. The standard curve was constructed by plotting the peak height ratio of each standard against its respective concentration; the unknowns were measured by interpolation from the standard curve (Figure 2).

Sensitivity

All samples were assayed in duplicate and results were calculated where the inhibition was within the range 15-80%. Samples with

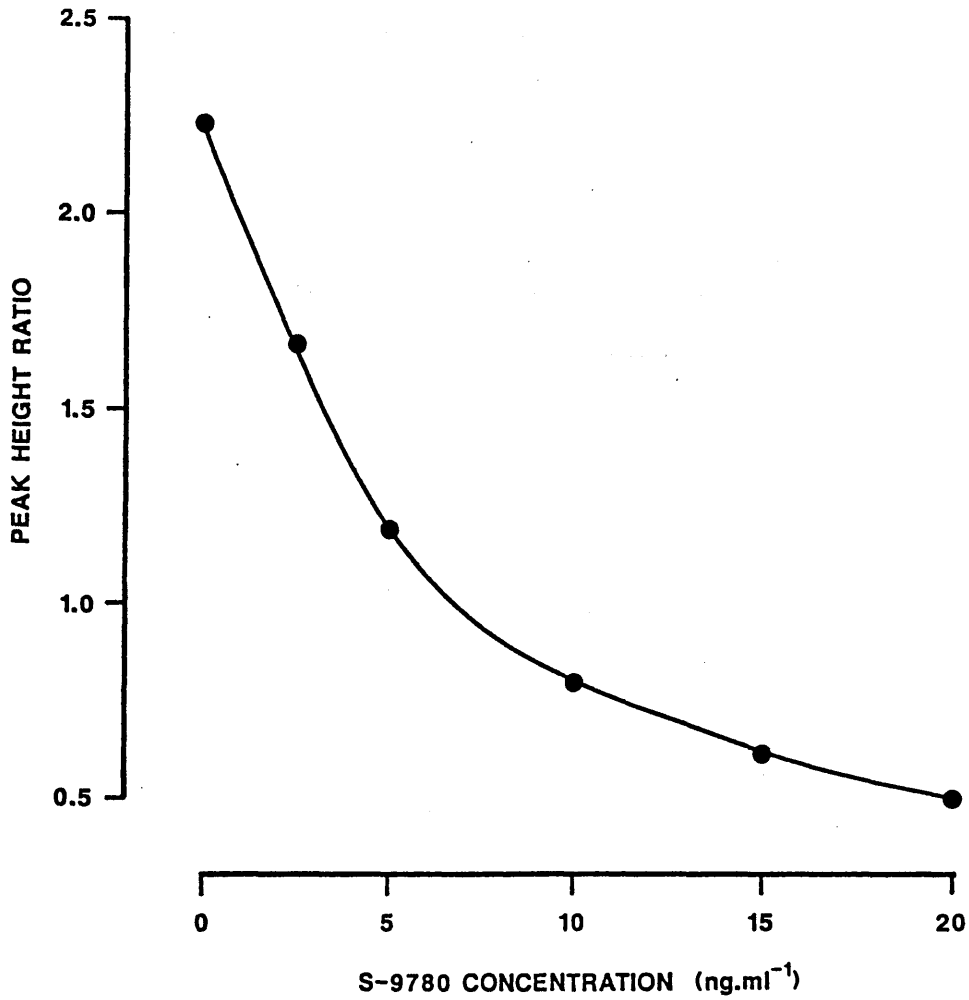


Figure 2. Typical standard curve for S-9780 assay.

inhibition greater than 80% (< 25 $ng.ml^{-1}$) were reanalysed with 1:1 dilution of the plasma. Inhibition less than 15% was defined as being below the limit of sensitivity of the assay, and in the assays performed, this was within the range of 0.5 to 1.0 $ng.ml^{-1}$.

Specificity

The specificity of the assay was assessed with reference to the parent drug compound, perindopril. Plasma samples were spiked with 1000 $ng.ml^{-1}$ of perindopril and were run through the assay using both rabbit and rat plasma. No inhibition was observed in the final enzyme assay step.

Further studies were undertaken to consider the assay specificity, however, by incubation of the XAD-4 column eluate with dilute rabbit and rat plasma prior to addition of angiotensin converting enzyme substrate. It was apparent from this study that with incubation times in excess of 6 hours rat plasma induced approximately 20% inhibition in the final assay step, indicating conversion of perindopril to S-9780. In contrast, no inhibition was observed with rabbit plasma. As a consequence, rabbit plasma was used in all assays.

Reproducibility

Intra assay variability was assessed by the replicate analysis of 'spiked' plasma standards both within the normal range of the assay and, after dilution, beyond the upper limit of the assay. The results are summarised in Table 1.

Inter assay reproducibility was assessed by the use of quality

Plasma standard $ng.ml^{-1}$	mean $ng.ml^{-1}$	SD	CV %	n
1	1.1	0.2	16.0	12
5	5.2	0.5	10.4	10
10	9.9	0.7	7.3	10
15	16.3	1.0	6.4	10
20	18.0	1.5	8.4	13
200	189.9	29.2	15.4	13

Table 1. Plasma S-9780 estimation: intra assay precision and variability. Samples having concentrations greater than 20 $ng.ml^{-1}$ were assayed after appropriate dilution.

control standards which were run with each assay, at concentrations of 4.0 $ng.ml^{-1}$ and 16.0 $ng.ml^{-1}$. The coefficient of variation for the lower standard was 8.8% and for the upper standard was 4.1% (n = 29).

CHAPTER 3

ORAL DOSE RANGING STUDY WITH PERINDOPRIL

3.1 INTRODUCTION

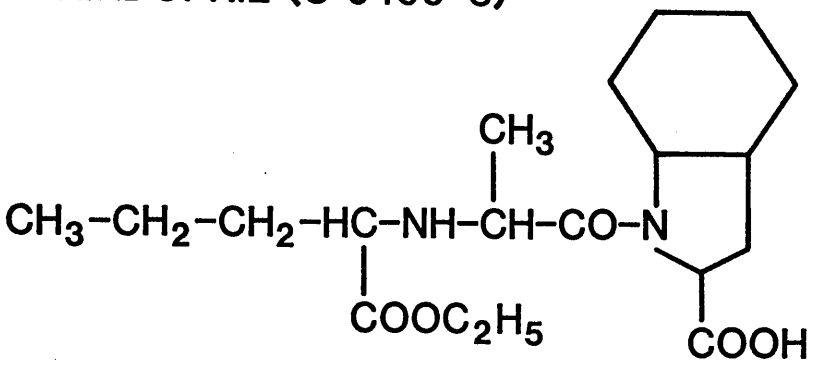
Perindopril is a new orally active inhibitor of angiotensin converting enzyme. It has a non thiol structure (Figure 3) and although it is itself relatively inactive, it is rapidly hydrolysed *in vivo* to the active diacid metabolite, S-9780. This compound is a potent and long lasting inhibitor of angiotensin converting enzyme in animals (116) and in preliminary single dose studies in man (Institut de Recherches Internationales Servier: personal communication). This chapter describes a study of the tolerance and dynamic effects of oral perindopril for 7 days in healthy normotensive volunteers.

3.2 METHODS

Subjects

Thirty six male volunteers were recruited from the staff of Stobhill General Hospital. They were screened prior to entry by history, general examination, urinalysis, electrocardiograph and routine laboratory tests of haematology and serum biochemistry to ensure good health. Two further volunteers were rejected, one because of mild iron deficiency and the other because he was found to have Gilbert's disease. Written informed consent was obtained from all subjects and

PERINDOPRIL (S 9490-3)



(S 9780)

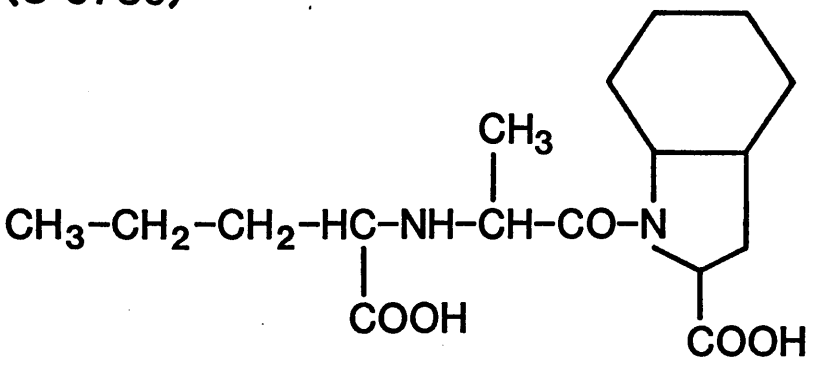


Figure 3. Chemical structure of perindopril and of its active diacid metabolite, S-9780.

the study design was approved by the local ethical review committee. The mean age of the volunteers was 25 years (range 18 - 35), their mean weight was 72 kg (range 59 - 92) and their mean height was 176 cm (range 162 - 188). Full details are given in Table 2(a-c). There was no statistical difference between the sub-groups. No salt restriction was imposed and although salt status was not measured on this occasion, subsequent experience with a group of these subjects showed 24 hour urinary sodium excretion to be 226 mmol (range 124 - 406, n = 8) and 24 hour urinary potassium excretion to be 82 mmol (range 10 - 141, n = 8).

Study Design

This was an open, dose ranging study in 6 parallel groups; blocked randomisation of 12 subjects to either placebo or 8 mg of perindopril was carried out, however, and these subjects were studied in double blind fashion. The other doses studied were 1, 2, 4 and 16 mg. Each dose was administered for seven consecutive days to 6 subjects.

The subjects arrived at the Clinical Pharmacology Research Unit at 0830 hours on the first study day. They had fasted since 2200 hours on the previous night and had avoided all drugs for the previous two weeks. No smoking, caffeine or alcohol ingestion was permitted during the study. An indwelling venous cannula was inserted in to an antecubital vein and the subjects rested supine until the start of the study. Blood pressure and heart rate were measured in duplicate by Sentron semiautomatic sphygmomanometer (Bard Biomedical) after ten minutes' supine rest and 5 minutes standing before dosing and at the following intervals after dosing: 1, 2, 4, 6, 8, 10, 12 and 24 hours. Blood samples were drawn for plasma angiotensin converting

Dose	Subject	Age years	Weight kg	Height cm
Placebo	20	22	65.9	172
	22	26	92.1	178
	23	30	71.0	174
	24	22	79.8	181
	27	27	67.6	162
	29	26	74.0	172
	mean	26	75.1	173
	SD	3	9.7	7
1 mg	5	24	62.7	173
	6	18	68.3	183
	11	31	71.4	180
	12	22	74.1	181
	13	34	72.0	167
	16	22	88.8	175
	mean	25	72.9	176
	SD	6	8.8	6

Table 2a. Demographic data for subjects studied after oral perindopril. Subject numbers are in order of recruitment and are referred to later in the text.

	Subject	Age years	Weight kg	Height cm
2 mg	1	22	65.8	176
	2	35	62.6	174
	3	27	62.0	184
	4	22	75.7	176
	7	22	69.4	178
	8	22	62.0	179
	mean	25	66.2	178
	SD	5	5.5	3
4 mg	9	26	67.5	172
	10	33	85.0	183
	14	30	64.5	168
	15	28	68.5	173
	17	28	67.5	170
	18	34	65.5	178
	mean	30	69.8	174
	SD	3	7.6	6

Table 2b. Demographic data (continued).

Dose	Subject	Age	Weight	Height
		years	kg	cm
8 mg	19	28	72.3	178
	21	22	65.8	177
	25	21	71.4	168
	26	28	71.7	176
	28	31	82.4	180
	30	22	59.2	167
	mean	25	70.5	174
	SD	4	7.7	5
16 mg	31	19	79.4	173
	32	25	70.0	179
	33	21	67.0	178
	34	21	77.0	185
	35	22	76.0	188
	36	23	79.0	188
	mean	22	74.7	182
	SD	2	5.1	6

Table 2c. Demographic data (continued).

enzyme activity at the same times as blood pressure was measured. Samples were also collected for plasma renin activity, aldosterone, adrenaline and noradrenaline levels pre-dosing and after 4, 8 and 24 hours.

Plasma samples for assay of S-9780, the active diacid metabolite, were also collected and are described in Chapter 7.

Capsules containing the appropriate dose of drug or placebo were administered orally with 200 ml of water at 0900 hours and the subjects remained fasting for a further two hours. A light standard meal was provided after 4 hours and 8 hours. Free fluids were permitted after 4 hours and after this time the volunteers were no longer restricted to bed between recordings.

On the second to sixth days of dosing, the volunteers attended the laboratory at 0900 hours and 1500 hours for further blood pressure recordings and blood sampling. The drug was administered after the 0900 sample each day. The results of these recordings are not reported in this chapter.

On the seventh day of treatment, the protocol for the first day was repeated exactly. The volunteers were reviewed 24 and 48 hours after this final dose.

The pre-study screening investigations were repeated both during the week of the study and after completion, the subjects were questioned about any symptoms at each recording time, and samples for angiotensin converting enzyme activity were measured within 1 to 2

days for the initial subjects in order to minimise the risk of toxicity.

Laboratory methods

These have been described in Chapter 2.

Statistical Analysis

Repeated measures analysis of variance or covariance was undertaken to compare with placebo the effects of the different doses on blood pressure, heart rate, plasma angiotensin converting enzyme and renin activity, aldosterone, adrenaline and noradrenaline levels. The pretreatment blood pressure and pretreatment aldosterone levels were taken as covariates in order to limit the variation caused by examining parallel groups. Where appropriate, confidence intervals were constructed using Dunnet's 't' statistic (54) for the difference between treated groups and placebo, making allowance for the multiple comparisons. The plasma hormone data were transformed to logarithms before analysis to normalise the distribution.

3.3 RESULTS

Tolerance

The drug was well tolerated by all subjects. Headache was frequently observed during both active treatment and placebo. Lightheadedness was noted with some intermediate doses but was not related to any postural fall in blood pressure. These symptoms were nearly all confined to the first and seventh days of treatment: the days of intensive recordings. One subject complained of impotence during

treatment with 4 mg daily. This resolved on completion of the study. There was no change in the screening tests either during or after treatment in any subject.

Angiotensin Converting Enzyme Activity

Perindopril inhibited plasma angiotensin converting enzyme in a dose related manner ($p < 0.001$, Figure 4). Sixteen mg daily produced about 90% inhibition 4 hours after dosing, with a fairly steady level of more than 80% inhibition beyond 1 to 12 hours after dosing on chronic once daily treatment. Even 24 hours after dosing, approximately 60% inhibition persisted with 8 mg and 16 mg daily. An increase in inhibition on the seventh day compared with the first was only noted with 1 mg, 2 mg and 4 mg daily ($p < 0.001$). Table 3 displays the angiotensin converting enzyme inhibition as mean $\pm$ SD for each time and dose.

Plasma Renin Activity

Four mg or more of perindopril raised plasma renin activity after the first dose ($p < 0.001$). A rise was also noted with 1 mg and 2 mg but the baseline renin was different in these two groups. All doses, 1 to 16 mg, caused a further rise in renin after 7 days of treatment (Figure 4). The area under the curve for plasma renin activity (after logarithmic transformation) clearly shows a dose-related response (Figure 5). After 7 days of treatment, 4 mg and over maintained the renin above the normal range (4 - 12 $ngAI.ml^{-1}.hr^{-1}$) even 24 hours after dosing. The median renin activity and 95% confidence limits for each time and dose are shown in Table 4.

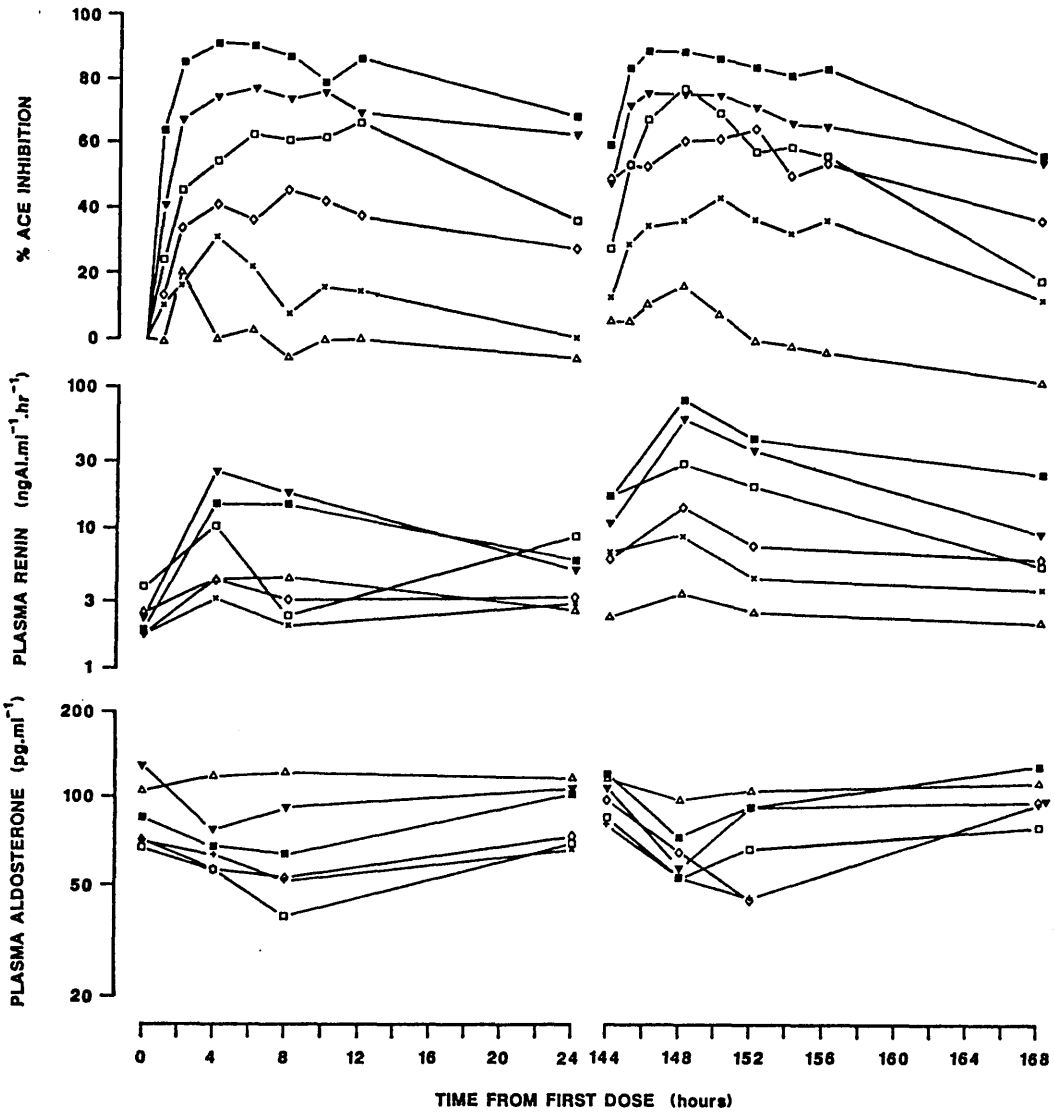


Figure 4. Plasma angiotensin converting enzyme inhibition, renin activity and aldosterone concentrations following the first and seventh daily administrations of placebo (△) and of 1 mg (✖), 2 mg (◇), 4 mg (□), 8 mg (▼) and 16 mg (■) of perindopril orally. The effects were significant by analysis of variance: ACE inhibition ($p < 0.001$); renin activity ($p < 0.001$); and aldosterone ($p < 0.05$). Each point represents the mean for six subjects.

	Placebo		1 mg		2 mg	
Time	Mean	SD	Mean	SD	Mean	SD
0	0.0	0.0	0.0	0.0	0.0	0.0
1	-1.5	5.5	10.2	18.6	13.9	21.0
2	18.5	24.1	16.2	21.1	34.3	18.3
4	0.6	11.2	31.2	26.2	41.3	23.9
6	3.4	10.4	22.2	26.6	36.7	25.5
8	-5.0	14.4	8.0	28.5	46.2	22.1
10	0.3	12.5	16.3	16.8	42.7	19.3
12	0.7	17.4	15.0	19.4	38.0	25.2
24	-4.6	9.6	1.0	25.7	28.6	29.3
144	6.6	9.4	13.1	29.6	49.8	20.1
145	6.4	17.7	29.5	24.0	54.5	18.8
146	11.9	32.8	35.5	11.9	53.8	21.0
148	17.4	32.1	37.1	12.0	61.8	27.0
150	8.6	22.8	44.1	9.3	62.5	23.6
152	0.5	9.5	37.4	19.6	65.7	18.6
154	-1.1	11.4	33.1	19.9	51.2	27.5
156	-3.1	15.7	37.1	13.4	55.0	23.4
168	-12.1	8.9	12.9	32.1	37.5	27.2

Table 3a. Plasma angiotensin converting enzyme inhibition following oral perindopril, expressed as 100 - (ACE/pretreatment ACE), n = 6.

	4 mg		8 mg		16 mg	
Time	Mean	SD	Mean	SD	Mean	SD
0	0.0	0.0	0.0	0.0	0.0	0.0
1	24.4	16.1	40.8	41.9	64.2	14.6
2	45.7	15.6	67.5	27.0	85.6	7.6
4	54.9	21.3	74.6	11.9	91.1	4.1
6	63.0	32.7	77.0	12.0	90.5	3.5
8	61.5	8.1	74.0	13.1	87.2	3.2
10	62.2	11.0	76.1	17.2	79.1	5.7
12	66.5	5.1	69.3	17.9	86.8	6.3
24	37.1	4.2	63.5	14.7	69.0	6.5
144	28.6	16.8	48.0	14.7	60.2	10.5
145	53.7	20.4	72.5	9.3	84.2	7.6
146	68.5	14.3	76.4	8.5	89.6	3.8
148	78.1	14.4	76.4	12.2	89.4	5.6
150	70.4	13.6	76.0	12.5	87.3	3.4
152	58.3	10.5	72.2	12.8	84.5	4.5
154	59.9	13.0	67.0	8.0	82.1	4.6
156	57.0	14.8	66.4	9.6	84.1	2.7
168	19.2	20.6	55.3	16.4	57.3	14.7

Table 3b. Plasma angiotensin converting enzyme inhibition, continued.

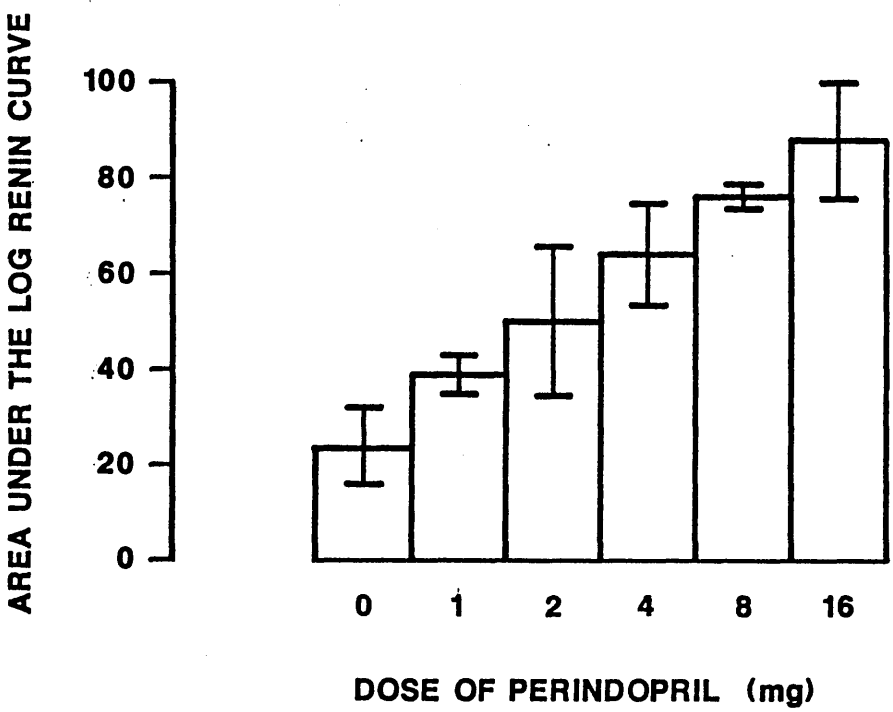


Figure 5. Areas under the curve for logarithm of plasma renin activity on the seventh day of treatment (mean $\pm$ SD, n = 6). Units are given by hours x ln ($ngAI.ml^{-1}.hr^{-1}$).

		Plasma renin activity $ngAI.ml^{-1}.hr^{-1}$		Plasma aldosterone $pg.ml^{-1}$	
	Time	Median	95% limits	Median	95% limits
Placebo	0	2.5	1.9 - 3.1	130	116 - 146
	4	6.6	5.1 - 8.4	93	82 - 105
	8	4.5	3.5 - 5.7	102	91 - 115
	24	2.1	1.6 - 2.6	132	117 - 148
	144	1.5	1.2 - 1.9	133	118 - 149
	148	4.8	3.8 - 6.1	96	86 - 108
	152	2.7	2.1 - 3.4	89	79 - 100
	168	2.7	2.1 - 3.4	127	113 - 142
1 mg	0	1.5	1.2 - 1.9	72	64 - 81
	4	4.2	3.3 - 5.3	54	48 - 60
	8	2.8	2.2 - 3.6	57	50 - 64
	24	2.0	1.5 - 2.5	66	58 - 74
	144	3.8	3.0 - 4.8	75	66 - 84
	148	11.3	8.9 - 14.4	52	46 - 58
	152	6.5	5.1 - 8.3	50	44 - 56
	168	4.2	3.2 - 5.5	79	70 - 91

Table 4a. Plasma renin activity and aldosterone levels following oral perindopril. As the data were analysed after logarithmic transformation, the median and 95% confidence limits for each time are shown, n = 6. Renin was raised by active treatment ($p < 0.001$) and aldosterone was depressed ($p < 0.05$). Normal ranges for renin and aldosterone are 4 - 12 $ngAI.ml^{-1}.hr^{-1}$ and 12 - 125 $pg.ml^{-1}$ respectively.

	Time	Plasma renin activity $ngAI.ml^{-1}.hr^{-1}$ Median	95% limits	Plasma aldosterone $pg.ml^{-1}$ Median	95% limits
2 mg	0	2.1	1.6 - 2.6	70	62 - 78
	4	5.1	4.0 - 6.5	49	44 - 55
	8	3.5	2.8 - 4.5	62	55 - 70
	24	2.2	1.7 - 2.8	68	60 - 76
	144	4.7	3.7 - 6.0	89	79 - 100
	148	16.2	12.8 - 20.5	65	58 - 73
	152	9.0	7.1 - 11.5	52	47 - 59
	168	6.5	5.1 - 8.3	89	79 - 100
4 mg	0	3.3	2.6 - 4.3	65	58 - 73
	4	10.3	8.1 - 13.1	48	43 - 55
	8	3.5	2.8 - 4.5	40	35 - 45
	24	6.1	4.6 - 8.1	73	64 - 82
	144	10.1	7.9 - 12.8	77	68 - 86
	148	28.0	22.0 - 35.6	54	48 - 60
	152	31.4	24.7 - 39.9	66	58 - 75
	168	8.2	6.1 - 11.0	67	58 - 77

Table 4b. Plasma renin activity and aldosterone levels, continued.

		Plasma renin activity $ngAI.ml^{-1}.hr^{-1}$		Plasma aldosterone $pg.ml^{-1}$	
	Time	Median	95% limits	Median	95% limits
8 mg	0	5.0	3.9 - 6.3	125	111 - 140
	4	17.2	13.5 - 21.8	86	76 - 96
	8	11.1	8.7 - 14.0	79	70 - 89
	24	6.3	5.0 - 8.1	114	101 - 128
	144	15.6	12.3 - 19.7	96	85 - 108
	148	38.7	30.5 - 49.0	72	64 - 81
	152	23.1	18.2 - 29.2	79	71 - 89
	168	18.1	14.3 - 23.0	101	90 - 114
16 mg	0	4.1	3.2 - 5.2	92	82 - 103
	4	12.9	10.2 - 16.3	67	60 - 76
	8	10.5	8.3 - 13.3	62	55 - 70
	24	4.8	3.8 - 6.1	92	81 - 103
	144	25.0	19.7 - 31.7	119	106 - 133
	148	67.9	53.5 - 86.1	84	75 - 94
	152	32.1	25.3 - 40.7	92	82 - 103
	168	31.4	24.8 - 39.9	115	103 - 129

Table 4c. Plasma renin activity and aldosterone levels, continued.

Plasma Aldosterone

The pretreatment aldosterone levels in several treatment groups differed from those in the placebo group. Correction for this difference was made initially by expressing the results as a percentage of the pretreatment level before analysis. The variation in the results obtained concealed any significant difference which may have been present (120). Subsequent examination of the raw data by analysis of covariance revealed a treatment effect ($p = 0.034$) and treatment-time interaction ($p = 0.01$). The data are displayed in Figure 4 and are shown more fully in Table 4.

Plasma Adrenaline

Perindopril had no effect on the plasma adrenaline levels at the times of sampling ($p = 0.59$, Table 5).

Plasma Noradrenaline

The plasma noradrenaline levels were not altered by active treatment ($p = 0.99$). Plasma catecholamine values are shown in Table 5.

Blood Pressure

No significant change in systolic blood pressure or supine diastolic blood pressure was detected. Erect diastolic blood pressure fell with active treatment ($p = 0.023$), particularly with 16 mg of perindopril. Supine heart rate was not altered by active treatment ($p = 0.13$), but a treatment-time interaction was present for erect heart rate ($p = 0.017$). Comparison of the heart rates at individual times after dosing reveals that perindopril 16 mg daily caused a significant rise in heart rate only in one recording after one week's treatment. The erect blood pressure and heart rate data are

Dose	Time	Plasma noradrenaline nmol.l^{-1}		Plasma adrenaline nmol.l^{-1}	
		Median	95% limits	Median	95% limits
Placebo	0	0.7	0.4 - 1.2	0.4	0.1 - 1.1
	4	0.7	0.3 - 1.9	0.4	0.1 - 0.9
	8	0.7	0.3 - 1.3	0.2	0.1 - 0.4
	24	0.9	0.4 - 2.1	0.3	0.1 - 0.8
	144	0.7	0.3 - 1.6	0.4	0.1 - 2.0
	148	0.9	0.5 - 1.7	0.3	0.2 - 0.6
	152	0.7	0.3 - 1.7	0.2	0.1 - 0.3
	168	0.9	0.4 - 2.0	0.3	0.1 - 0.6
1 mg	0	1.6	1.1 - 2.2	0.3	0.1 - 0.6
	4	1.7	1.2 - 2.5	0.2	0.1 - 0.2
	8	1.6	1.1 - 2.3	0.2	0.1 - 0.4
	24	1.8	1.5 - 2.2	0.1	0.0 - 0.4
	144	2.0	1.3 - 3.2	0.2	0.1 - 0.7
	148	1.9	1.2 - 3.0	0.2	0.1 - 0.4
	152	1.5	1.0 - 2.1	0.1	0.1 - 0.3
	168	1.6	1.3 - 2.0	0.3	0.2 - 0.4

Table 5a. Plasma catecholamines after oral perindopril, n = 6. The data were analysed after logarithmic transformation. There was no significant change from placebo (noradrenaline, p = 0.59; adrenaline, p = 0.99). Normal ranges are 0.3 - 7.5 nmol.l^{-1} and 0.0 - 1.0 nmol.l^{-1} for noradrenaline and adrenaline respectively.

Dose	Time	Plasma noradrenaline nmol.l^{-1}		Plasma adrenaline nmol.l^{-1}	
		Median	95% limits	Median	95% limits
2 mg	0	2.0	1.0 - 4.3	0.3	0.2 - 0.4
	4	2.0	1.5 - 2.7	0.2	0.1 - 0.4
	8	1.3	1.0 - 1.7	0.1	0.1 - 0.3
	24	1.7	1.3 - 2.2	0.2	0.1 - 0.3
	144	2.7	1.9 - 3.8	0.2	0.1 - 0.2
	148	2.0	1.4 - 2.9	0.2	0.1 - 0.3
	152	1.5	0.9 - 2.6	0.1	0.1 - 0.4
	168	2.2	1.4 - 3.5	0.2	0.1 - 0.4
4 mg	0	1.6	1.2 - 2.2	0.3	0.2 - 0.5
	4	1.7	1.3 - 2.2	0.1	0.0 - 0.4
	8	1.5	1.1 - 2.2	0.2	0.1 - 0.3
	24	2.1	1.4 - 3.1	0.3	0.2 - 0.4
	144	1.9	1.6 - 2.4	0.2	0.1 - 0.4
	148	2.0	1.8 - 2.2	0.1	0.0 - 0.4
	152	2.2	1.4 - 3.2	0.2	0.1 - 0.4
	168	2.8	2.0 - 4.0	0.3	0.2 - 0.4

Table 5b. Plasma catecholamines (continued).

		Plasma noradrenaline nmol.l^{-1}		Plasma adrenaline nmol.l^{-1}	
Dose	Time	Median	95% limits	Median	95% limits
8 mg	0	1.1	0.6 - 2.4	0.6	0.2 - 1.8
	4	1.4	0.7 - 2.9	0.4	0.3 - 0.7
	8	1.2	0.6 - 2.4	0.3	0.2 - 0.5
	24	1.1	0.4 - 2.8	0.4	0.2 - 0.8
	144	1.1	0.5 - 2.2	0.5	0.2 - 1.1
	148	1.1	0.4 - 2.7	0.3	0.2 - 0.4
	152	1.0	0.5 - 2.1	0.2	0.1 - 0.5
	168	1.5	0.4 - 5.2	0.3	0.2 - 0.7
16 mg	0	1.8	1.4 - 2.4	0.9	0.5 - 1.5
	4	2.3	1.8 - 3.0	0.6	0.3 - 1.1
	8	1.3	0.7 - 2.4	0.4	0.2 - 0.9
	24	2.4	1.7 - 3.4	0.7	0.3 - 1.5
	144	2.1	1.5 - 2.9	0.5	0.2 - 1.1
	148	2.3	1.9 - 2.7	0.6	0.2 - 1.7
	152	1.9	1.4 - 2.6	0.4	0.2 - 1.4
	168	2.5	1.8 - 3.5	0.7	0.4 - 1.2

Table 5c. Plasma catecholamines (continued).

displayed in Figure 6 and the 95% confidence intervals for a difference between perindopril-treated and placebo groups are given in Tables 6 to 8.

3.4 DISCUSSION

Perindopril was well tolerated in doses of up to 16 mg daily for seven days. The incidence of minor symptoms was similar in all groups, including placebo. Although impotence occurred in one subject taking 4 mg, this cannot with certainty be attributed to the drug. No untoward effect was found on routine physical examination or laboratory screening. The effects of perindopril on plasma angiotensin converting enzyme were dose-dependent in the range studied. With 16 mg, peak inhibition of 90% was achieved and significant inhibition persisted, around 60%, 24 hours after dosing with 8 mg or 16 mg. As would be expected, plasma renin activity was elevated by active treatment. Doses of 4 mg and over caused the plasma renin activity to be maintained above the normal range.

Despite the profound elevation of plasma renin activity, the reduction in plasma aldosterone levels was small and variable. The normal range for aldosterone is wide, however, and the parallel group design of the study made detection of changes in aldosterone relatively difficult.

Perindopril clearly lowered erect diastolic blood pressure. It may be difficult to demonstrate hypotensive activity in normal subjects, particularly when no salt restriction is imposed. Thus, the lack of

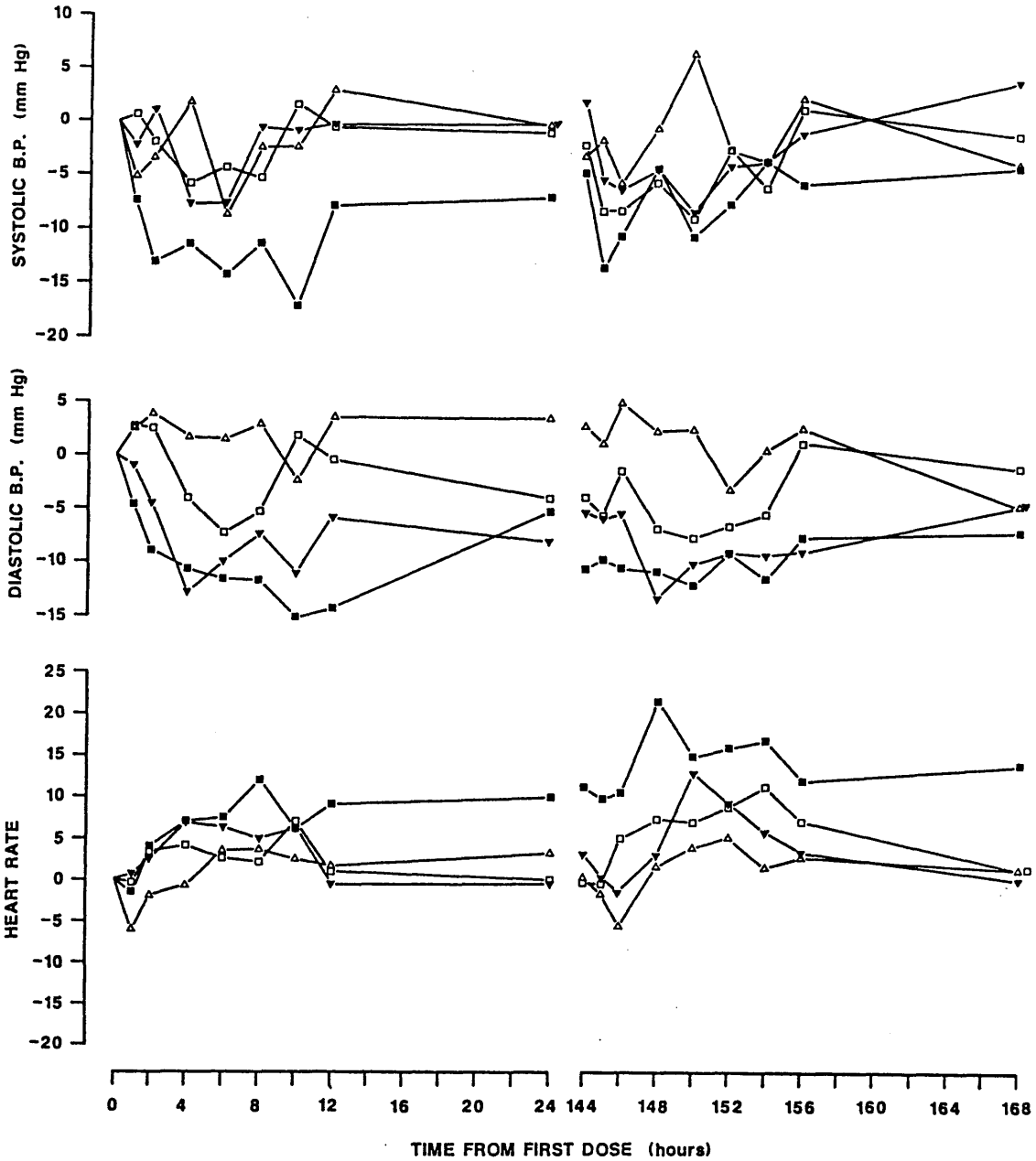


Figure 6. Standing blood pressure and heart rate following the first and seventh daily administrations of placebo (△) and 4 mg (□), 8 mg (▼) and 16 mg (■) of perindopril orally; the data for 1 mg and 2 mg are omitted for clarity. The effects on diastolic blood pressure were significant by analysis of variance ($p < 0.05$) but systolic pressure did not change ($p = 0.13$). A time-treatment interaction for heart rate was present ($p < 0.05$).

Time	1 mg		2 mg		4 mg		8 mg		16 mg	
1	-9.9	16.9	-10.2	16.6	-7.6	19.2	-10.6	16.2	-15.7	11.1
2	-14.4	12.2	-11.6	15.0	-11.8	14.8	-8.8	17.8	-23.0	3.6
4	-14.4	5.2	-16.9	2.7	-17.6	2.0	-19.5	0.1	-23.1	-3.5
6	-3.6	22.9	-8.6	17.9	-8.9	17.6	-12.3	14.2	-18.8	7.7
8	-10.2	16.2	-13.2	13.2	-16.1	10.2	-11.4	14.9	-22.1	4.2
10	-13.0	19.3	-15.2	17.2	-12.3	20.1	-14.8	17.5	-30.9	1.4
12	-16.3	20.0	-21.2	15.2	-21.8	14.5	-21.5	14.8	-29.1	7.3
24	-13.8	9.6	-15.8	7.6	-12.4	10.9	-11.6	11.8	-18.3	5.0
144	-15.4	14.4	-15.1	14.7	-13.8	16.0	-9.8	20.0	-16.4	13.4
145	-17.8	8.9	-19.6	7.1	-20.1	6.6	-17.3	9.4	-25.3	1.4
146	-13.5	17.2	-18.9	11.9	-17.9	12.8	-15.9	14.8	-20.2	10.5
148	-21.0	9.9	-23.9	6.9	-20.5	10.4	-19.2	11.7	-19.3	11.6
150	-20.0	8.2	-28.8	-0.7	-29.8	-1.7	-29.7	-1.5	-31.5	-3.3
152	-20.7	11.4	-23.3	8.8	-16.0	16.0	-17.5	14.5	-21.1	11.0
154	-10.3	10.8	-10.5	10.7	-13.1	8.1	-10.7	10.5	-10.8	10.3
156	-16.5	13.8	-19.6	10.8	-16.3	14.1	-18.6	11.8	-23.4	6.9
168	-18.2	15.4	-12.9	20.7	-14.2	19.5	-9.1	24.6	-17.2	16.5

Table 6. Standing systolic blood pressure (mmHg): 95% confidence intervals for active - placebo, n = 6. The mean blood pressures shown in Figure 6.

Time	1 mg		2 mg		4 mg		8 mg		16 mg	
1	-13.8	9.7	-12.1	11.4	-11.3	12.2	-15.3	8.3	-18.8	4.7
2	-16.7	10.7	-19.3	8.1	-15.2	12.2	-22.2	5.2	-26.6	0.8
4	-14.6	4.4	-15.8	3.2	-15.3	3.8	-24.1	-5.1	-21.8	-2.8
6	-18.8	6.8	-21.1	4.6	-21.7	4.0	-24.3	1.3	-25.9	-0.3
8	-12.4	14.3	-19.9	6.8	-21.6	5.1	-23.6	3.1	-27.9	-1.2
10	-7.1	18.6	-15.7	10.0	-8.7	17.0	-21.6	4.1	-25.5	0.2
12	-11.8	15.0	-19.5	7.3	-17.5	9.3	-22.9	3.9	-31.3	-4.5
24	-14.8	11.4	-17.9	8.3	-20.6	5.6	-24.6	1.6	-21.8	4.3
144	-16.7	11.1	-21.5	6.3	-20.6	7.1	-22.1	5.7	-27.4	0.4
145	-15.0	6.7	-15.0	6.7	-17.6	4.1	-18.0	3.7	-21.8	-0.1
146	-21.6	3.1	-22.8	1.8	-18.8	5.8	-22.9	1.7	-28.0	-3.4
148	-22.6	2.8	-22.8	2.5	-21.9	3.4	-28.5	-3.2	-25.9	-0.6
150	-21.0	3.4	-22.4	2.0	-22.5	2.0	-24.9	-0.5	-26.9	-2.5
152	-14.5	11.8	-16.5	9.7	-16.7	9.5	-19.0	7.2	-19.2	7.0
154	-21.0	7.2	-20.2	8.0	-20.3	7.8	-24.1	4.1	-26.2	1.9
156	-19.9	12.2	-22.4	9.7	-17.5	14.5	-27.8	4.3	-26.4	5.7
168	-6.1	16.1	-5.6	16.6	-7.5	14.8	-11.0	11.2	-13.5	8.8

Table 7. Standing diastolic blood pressure (mmHg): 95% confidence intervals for active - placebo, n = 6. The mean blood pressures are shown in Figure 6.

Time	1 mg		2 mg		4 mg		8 mg		16 mg	
1	-9.5	14.3	-13.6	10.2	-6.2	17.6	-5.3	18.5	-7.6	16.2
2	-9.6	16.1	-22.5	3.2	-7.7	18.0	-8.5	17.2	-6.9	18.8
4	-7.7	27.4	-17.5	17.7	-12.7	22.4	-10.0	25.2	-9.7	25.4
6	-12.4	17.3	-12.1	17.6	-15.8	13.9	-12.1	17.6	-11.0	18.7
8	-13.8	28.1	-20.7	21.2	-22.5	19.4	-19.7	22.2	-12.7	29.2
10	-17.4	16.9	-16.0	18.3	-12.7	21.7	-13.7	20.7	-13.5	20.8
12	-14.3	20.6	-21.9	12.9	-18.2	16.7	-19.8	15.1	-10.0	24.9
24	-11.3	13.5	-16.4	8.4	-15.7	9.2	-16.2	8.7	-5.7	19.2
144	-17.4	17.2	-20.7	13.9	-17.9	16.7	-14.4	20.2	-6.4	28.2
145	-18.0	16.0	-23.5	10.5	-15.7	18.2	-15.0	18.9	-5.4	28.5
146	-9.6	23.8	-17.6	15.8	-6.0	27.3	-12.8	20.6	-0.5	32.8
148	-5.4	23.9	-15.3	14.0	-8.9	20.4	-13.4	15.9	5.2	34.5
150	-15.4	15.3	-12.4	18.3	-12.3	18.4	-6.5	24.2	-4.4	26.3
152	-16.5	13.9	-19.6	10.8	-11.6	18.8	-11.2	19.2	-4.4	25.9
154	-20.3	16.8	-22.1	15.1	-8.8	28.3	-14.5	22.6	-3.3	33.8
156	-17.6	16.9	-18.1	16.4	-13.0	21.5	-16.9	17.6	-8.2	26.3
168	-13.0	26.5	-25.6	13.9	-19.9	19.6	-21.1	18.3	-7.1	32.4

Table 8. Standing heart rate (beats.min^{-1}): 95% confidence intervals for active - placebo, n = 6. The mean heart rates are shown in Figure 6.

change in supine pressure is not unexpected. Some increase in heart rate occurred after 7 days' treatment at the highest dose. This is in contrast to the reported effects of other angiotensin converting enzyme inhibitors (88,143,151) and perindopril acutely (Chapter 5). It may be that the large dose of perindopril caused volume depletion after several days' treatment and that this produced a reflex tachycardia.

The absence of any effect of perindopril on circulating catecholamine levels is consistent with results after enalapril (143).

The profile of angiotensin converting enzyme inhibition after perindopril is different to that seen after captopril (170) and enalapril (143): perindopril causes longer lasting inhibition for a given peak effect. Nevertheless, since enalapril is recognised to possess antihypertensive activity lasting for 24 hours or more after a 10 mg oral dose, when about 40% angiotensin converting enzyme inhibition persists (143), it appears reasonable to infer from our data with perindopril that a useful clinical dose will fall in the range 4 - 16 mg.

In conclusion, perindopril appears to be a safe and well tolerated inhibitor of plasma angiotensin converting enzyme in man. The effects on the renin angiotensin system and blood pressure are predictable. Further studies in volunteers and hypertensive patients are justified. An appropriate dose for these studies would appear to be of the order of 8 to 10 mg.

CHAPTER 4

EFFECTS OF INTRAVENOUS S-9780

4.1 INTRODUCTION

Perindopril is hydrolysed after absorption to its active diacid metabolite, S-9780. Some knowledge of the dynamic effects and pharmacokinetics of S-9780 is necessary for full interpretation and extrapolation of the studies of the effects of oral perindopril. This study was designed to gather this necessary data. Since S-9780 is poorly absorbed, intravenous administration was chosen. This study was the first occasion on which S-9780 was given intravenously to man.

4.2 METHODS

Subjects

Eight normotensive male volunteers were recruited after screening by history, physical examination, urinalysis, electrocardiogram and routine laboratory tests of haematology and serum biochemistry to ensure good health. Written informed consent was obtained from all subjects and the study design was approved by the local ethical committee. The mean age of the volunteers was 26 years (range 18 - 43), their mean weight was 72 kg (range 65 - 78) and their mean height was 181 cm (range 173 - 190) (Table 9). No salt restriction was imposed.

Subject	Age years	Height cm	Weight kg
1	18	190	78
2	23	185	73
3	23	190	76
4	21	184	77
5	23	178	70
6	30	173	67
7	29	176	72
8	43	173	65
Mean	26	181	72
SD	8	7	5

Table 9. Demographic data for the volunteers studied.

Study Design

This was a double blind comparison of three intravenous doses of S-9780 with placebo. The subjects were randomly allocated to the rows of a balanced latin square to determine order of treatment. Thus, each volunteer attended on four occasions at least a week apart, and was given placebo, 1 mg, 2 mg or 4 mg of S-9780 on each occasion.

The subjects arrived at the Clinical Pharmacology Research Unit at 0830 hours on each study day. They had fasted since 2200 hours on the previous night and had avoided all drugs for the previous two weeks. No smoking, caffeine or alcohol ingestion was permitted during the study. An indwelling venous cannula was inserted in to an antecubital vein and the subjects rested until the start of the study.

Blood pressure and heart rate were measured, in duplicate after 10 minutes' supine rest and 5 minutes standing, before and at the following intervals after dosing: 20, 40, 60, 80, 100 minutes, 2, 3, 4, 6, 8, 24 and 48 hours. Blood samples were drawn for plasma angiotensin converting enzyme activity before dosing and at the following intervals after dosing: 5, 10, 15, 20, 30, 40, 50, 60, 80, 100 minutes, 2, 3, 4, 6, 8, 24 and 48 hours. Samples were also collected for plasma renin activity, aldosterone, adrenaline and noradrenaline levels pre-dosing and after 4, 8 and 24 hours. Further samples were collected for assay of S-9780 in plasma and investigation of pharmacokinetic profiles. These results are presented in Chapter 7.

At 0900 hours, 2 ml of normal saline containing the appropriate amount of S-9780 was injected as an intravenous bolus to the contralateral arm to the cannula. The subjects remained fasting for a further 2 hours but were then given a light standard breakfast (two slices of toast and a glass of milk) followed after the 4 hour recording by a light lunch. Free fluids were permitted from this time. The volunteers remained seated or supine for the first 8 hours of each study period but were then allowed home.

The volunteers were questioned about any symptoms they experienced at each blood pressure recording time and the pre-study screening tests were repeated after completion of the study.

Laboratory Methods

These have been fully described in Chapter 2.

Statistical Analysis

An effect of treatment order on any of the parameters which were measured was excluded by repeated measures analysis of variance before subsequent analysis. Repeated measures analysis of variance was employed to compare with placebo the effects of the different doses of S-9780 on all of the parameters measured. The plasma hormone data were transformed to logarithmic values before analysis in order to normalise the distribution.

Where a significant treatment effect or time-treatment interaction was present ($p < 0.05$), the effects of the various doses were then compared with placebo at the individual times after dosing by using

Dunnett's method for comparison of multiple treatment means with a single control (54).

4.3 RESULTS

Tolerance and Safety

The drug was generally well tolerated. Three subjects were completely asymptomatic. One complained of headache after both 1 mg and 4 mg of S-9780, one subject had headache only after 1 mg and another subject had headache only after 4 mg of S-9780. The remaining two subjects felt tired after treatment with 1 mg and 2 mg of S-9780 respectively. There was no evidence of any toxicity on routine urinalysis or laboratory tests of haematology and biochemistry.

Plasma Angiotensin Converting Enzyme Activity

Each dose of S-9780 examined caused immediate and maximal inhibition of plasma angiotensin converting enzyme. There was no significant difference between the profiles of inhibition by 1, 2 or 4 mg (Figure 7).

Plasma Renin Activity

Intravenous S-9780 caused elevation of plasma renin activity ($p < 0.0001$), when measured at 4 and 8 hours after dosing (Table 10). After 24 hours, plasma renin activity had almost returned to normal.

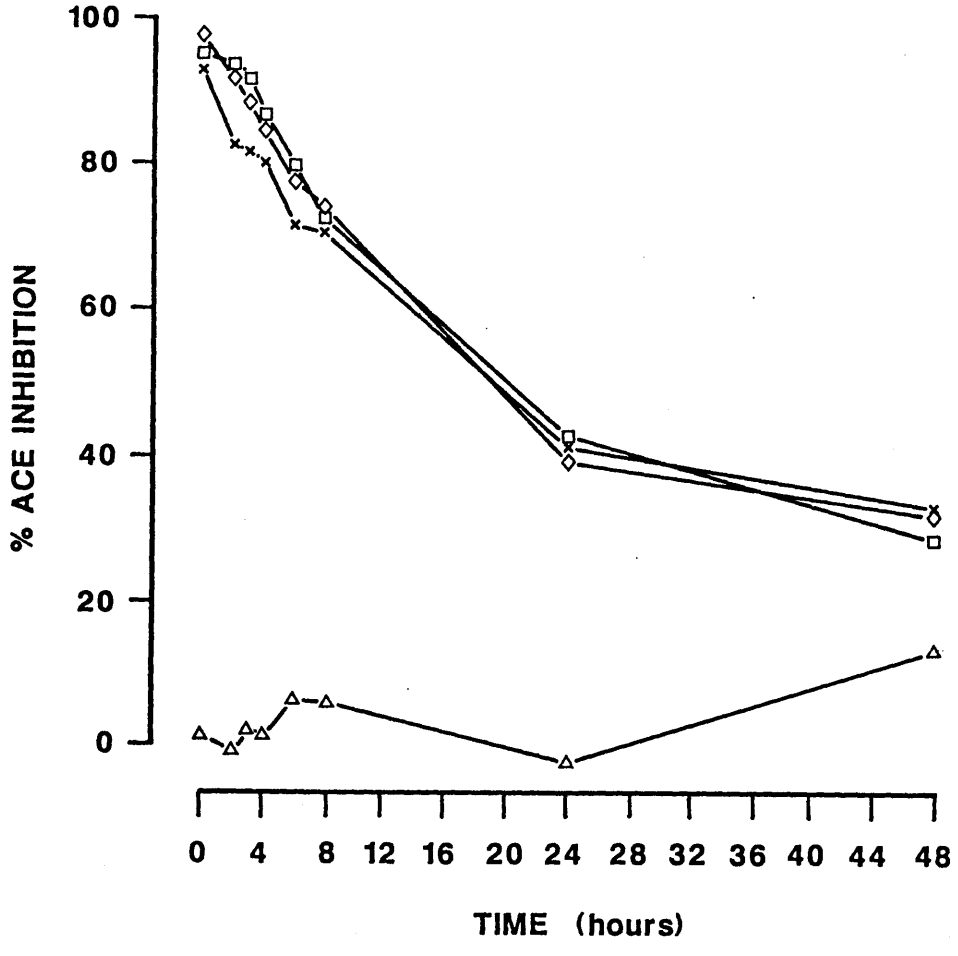


Figure 7. Inhibition of plasma angiotensin converting enzyme following acute intravenous administration of S-9780 1 mg (✖), 2 mg (◇) and 4 mg (□) and of placebo (△). There was no significant difference between the effects of the active doses.

		Plasma renin activity ngAI.ml^{-1}		Plasma aldosterone pg.ml^{-1}	
	hours	median	95% limits	median	95% limits
Placebo	0	2.2	1.4 - 3.5	103	60 - 177
	4	4.5	2.4 - 8.2	65	46 - 93
	8	2.5	2.1 - 3.1	60	37 - 97
	24	2.0	1.6 - 2.6		
1 mg	0	2.6	1.8 - 3.7	86	56 - 130
	4	14.1	4.8 - 41.3	59	28 - 128
	8	8.6	4.5 - 16.4	67	45 - 102
	24	3.9	2.7 - 5.7		
2 mg	0	1.9	1.4 - 2.7	89	71 - 112
	4	18.6	7.5 - 46.0	48	32 - 73
	8	8.0	4.6 - 14.2	57	37 - 87
	24	4.0	2.7 - 5.8		
4 mg	0	2.1	1.4 - 3.2	78	51 - 121
	4	35.1	30.3 - 40.8	56	40 - 80
	8	11.1	6.6 - 18.6	58	40 - 85
	24	5.0	3.0 - 8.5		

Table 10. Plasma renin activity and aldosterone levels after intravenous doses of S-9780. The data were analysed after logarithmic transformation, therefore the median and 95% confidence limits are used; n = 8 throughout. Analysis of variance confirmed significant changes after treatment for renin ($p < 0.0001$) but not for aldosterone ($p = 0.46$).

Plasma Aldosterone

No effect of treatment on plasma aldosterone levels was detected at the times of sampling (Table 10).

Plasma Catecholamines

No significant change in either plasma noradrenaline or adrenaline was detected after intravenous S-9780. The data are shown in Table 11.

Blood Pressure and Heart Rate

Erect diastolic blood pressure fell after intravenous S-9780 ($p < 0.01$). Within 20 minutes of dosing, 4 mg had lowered the diastolic pressure by 9 mmHg compared with placebo (95% confidence limits -17.1 to -0.7 mmHg) and the pressure was still at similar levels after 24 hours (95% confidence limits -16.8 to -0.5 mmHg). A trend towards lower values was also seen for erect systolic and supine blood pressures but these were not statistically significant. No significant change in heart rate occurred (Figure 8).

4.4 DISCUSSION

The volunteers tolerated intravenously administered S-9780 very well. The complaints of minor symptoms such as headache and tiredness were restricted to the days of active treatment but there was a total of only six complaints out of twenty four days when active treatment was administered and there was therefore no conclusive evidence that they were drug induced. In particular, there was no instance of symptomatic hypotension.

		Noradrenaline nmol.l^{-1}		Adrenaline nmol.l^{-1}	
	hours	median	95% limits	median	95% limits
Placebo	0	1.2	0.6 - 2.2	0.4	0.2 - 0.7
	4	2.1	1.3 - 3.4	0.4	0.3 - 0.6
	8	1.3	0.5 - 3.4	0.3	0.2 - 0.5
1 mg	0	0.7	0.3 - 1.4	0.4	0.2 - 0.7
	4	1.1	0.4 - 2.5	0.4	0.3 - 0.6
	8	0.8	0.4 - 1.4	0.3	0.2 - 0.5
2 mg	0	1.0	0.7 - 1.6	0.3	0.2 - 0.4
	4	1.4	1.0 - 2.0	0.2	0.2 - 0.4
	8	1.1	0.7 - 1.6	0.2	0.1 - 0.4
4 mg	0	1.0	0.5 - 2.0	0.3	0.2 - 0.5
	4	1.8	1.1 - 2.9	0.3	0.2 - 0.6
	8	1.7	1.0 - 2.9	0.4	0.3 - 0.6

Table 11. Plasma catecholamine levels after intravenous doses of S-9780. The data were analysed after logarithmic transformation, therefore the median and 95% confidence limits are displayed; n = 7 throughout. There was no significant change compared with placebo (noradrenaline, $p = 0.07$; adrenaline, $p = 0.21$).

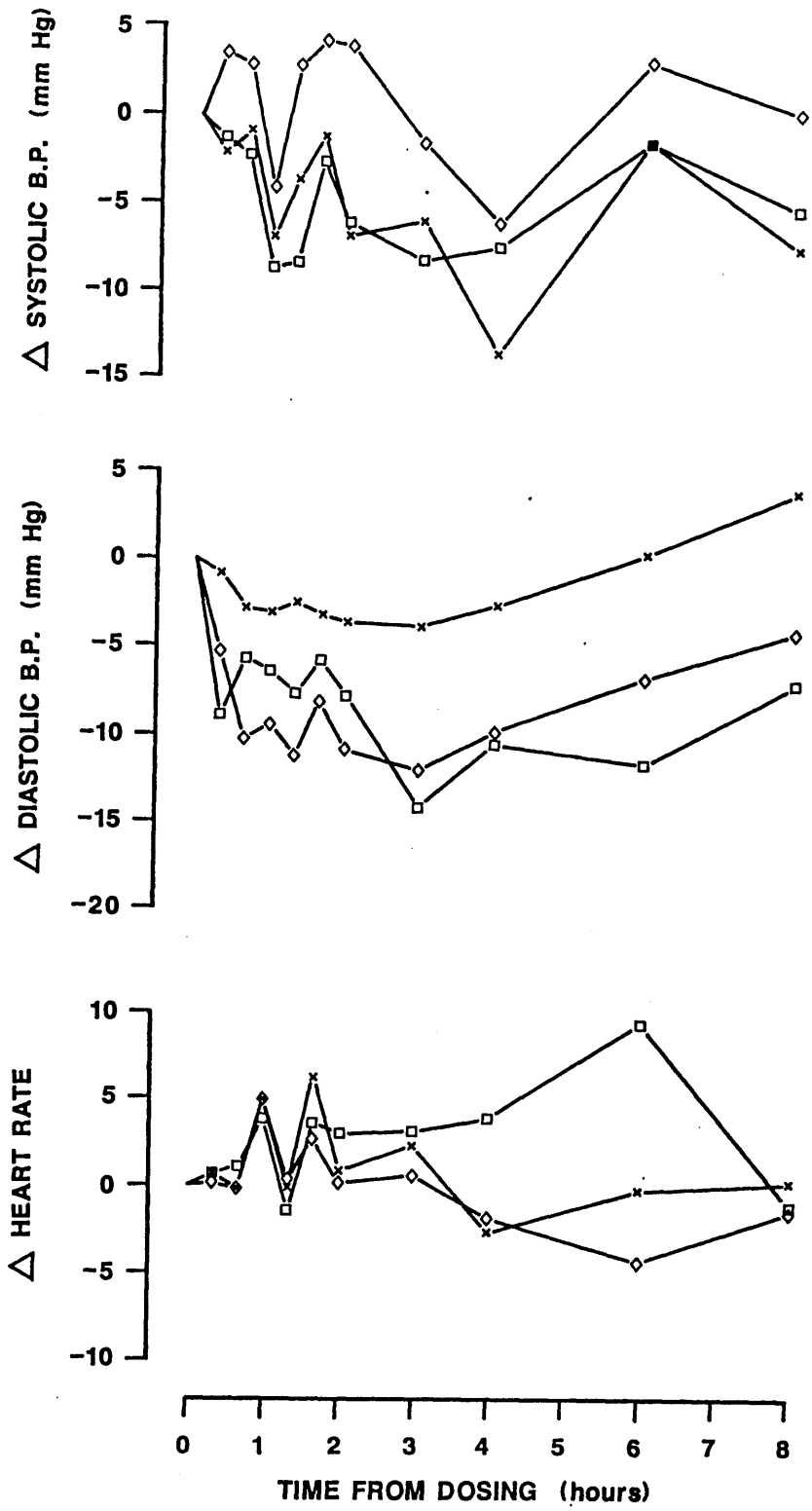


Figure 8. Mean standing blood pressure and heart rate after placebo correction following acute intravenous administration of 1 mg (✖), 2 mg (◇) and 4 mg (□) of S-9780, n = 8. Only the effects on diastolic pressure were significant by analysis of variance ($p < 0.01$).

The effects on blood pressure were modest but convincing. No salt restriction had been imposed and the subjects were normotensive, therefore a marked hypotensive response would not be expected. The lack of reflex tachycardia despite a fall in blood pressure provides further evidence that this is a feature of angiotensin converting enzyme inhibitors in general (88,143,151).

In view of the clear dose-related response of angiotensin converting enzyme to oral perindopril in doses of 1 to 16 mg (Chapter 3), it is slightly surprising that there was no detectable difference in the extent of enzyme inhibition following the various doses of S-9780. This suggests that even 1 mg of intravenously administered S-9780 has an effect at least as great as 16 mg of perindopril orally. Thus, it offers evidence that the bioavailability of the active metabolite is relatively low in man.

The response of plasma renin activity was dose related, however, with effects still detectable 24 hours after dosing. Despite this, the aldosterone levels did not change significantly although the confidence limits demonstrate that the power of this study to detect such a change was low.

In conclusion, S-9780 caused predictable changes in the renin angiotensin system and moderate lowering of blood pressure in normal salt replete volunteers. The drug was well tolerated and appears safe for further clinical study.

CHAPTER 5

AUTONOMIC EFFECTS OF PERINDOPRIL

5.1 INTRODUCTION

The preceding studies with perindopril have confirmed that it is a potent inhibitor of plasma angiotensin converting enzyme, with a long duration of action.

The withdrawal of the vasoconstrictor action of angiotensin II following angiotensin converting enzyme inhibition would result in arteriolar vasodilatation. The consistent reports of an absence of reflex tachycardia or sympathetic activation after converting enzyme inhibitors (88,143,151) are, however, at variance with the haemodynamic and neuro-endocrine responses to classical peripheral arterial vasodilators such as hydralazine (126).

This unusual haemodynamic profile has given rise to the concept that angiotensin converting enzyme inhibition may lead to consequences more complex than a simple removal of the vasoconstrictor role of angiotensin II. Specifically, a further effect of angiotensin II removal on autonomic function or baroreflex integrity has been suggested (210). Additionally, several other interpretations have been advanced, including an effect on venous capacitance (194), increased compliance of large arteries (121) and parasympathetic activation (2,27,188).

While enhanced cardiac vagal tone has been reported after captopril (27,188), enalapril (2) and lisinopril (2), it remains to be established whether this is a common property of angiotensin converting enzyme inhibitors or a specific feature of the individual drugs.

The aim of the present study was to examine the effects of perindopril on autonomic function and exercise performance, after single oral doses in healthy subjects.

5.2 METHODS

Subjects and Study Design

This was a double blind, randomised, placebo controlled, crossover study. Prior approval of the protocol was obtained from the local ethical review committee. Ten healthy, normotensive male subjects, aged 18 - 28 years and weighing 54 - 93 kg, gave written informed consent to participate in the study. The subjects were judged healthy by history, clinical examination and by biological (SMA-12), heamatological and electrocardiographic screening prior to entry to the study. Their sodium status was established by 24 hour urine collection: the mean excretion was 226 $\pm$ 95 mmol in 24 hours.

On two study days, two weeks apart, the volunteers attended the Clinical Pharmacology Research Unit at 0830 hours, 1 hour after a standard light breakfast having avoided coffee, tea, alcohol and cigarettes for 12 hours. They were allowed to rest for 30 minutes before the study commenced.

Placebo or 8 mg of perindopril in identical capsules was administered orally with 200 ml of water. Blood pressure and heart rate were recorded in duplicate after 10 minutes' supine rest and 1, 2 and 5 minutes standing, before and 1, 2, 4, 6 and 8 hours after dosing. A light snack was allowed after the two hour recording. Blood pressure was measured by a semiautomatic sphygmomanometer (Sentron; Bard Biomedical) and heart rate recorded by praecordial electrocardiogram electrodes connected to a Grass Polygraph model 7B 1B (Quincy; Mass., USA). Where required, heart periods were measured from the electrocardiograph tracing as the beat to beat intervals.

Autonomic Reflex Tests

The following tests of autonomic function were undertaken in the stated order between 4 and 5 hours after dosing, i.e. at the time of maximal drug effect. At least five minutes were allowed between each procedure or until haemodynamic variables returned to pre-manoeuvre levels, whichever was the longer.

1. Standing to lying test.

After 5 minutes of standing erect, subjects lay down on a bed. Heart rate was recorded over the last 10 beats before lying down and for 30 seconds after. The standing to lying ratio was calculated as the ratio of the mean erect heart period to the shortest heart period in the first 10 beats after starting to lie down (15).

2. Forearm isometric exercise.

Subjects were required to maintain 30% of pre-determined maximum hand grip on a modified sphygmomanometer for 2 minutes. Blood pressure and heart rate were measured before and at 1 minute intervals during exercise. The increase in blood pressure induced by the test is due to sympathetic stimulation.

3. Valsalva's manoeuvre.

The Valsalva's manoeuvre was performed in duplicate. In the supine position subjects maintained forced expiration to hold a column of mercury in a modified sphygmomanometer at 50 mmHg for 15 seconds. The electrocardiograph was continuously recorded. Blood pressure was measured before the manoeuvre and after termination of expiration, which coincides with the pressure overshoot (178). The difference between pre and post Valsalva blood pressures, and the ratio of the longest heart period during the manoeuvre to the shortest after, were used in the data analysis.

4. Diving reflex.

Volunteers sat for 5 minutes with faces held 3-4 cm above a basin of water at 18-20 C. On instruction, without taking a deep inspiration, they lowered their face into the water and remained immersed for as long as was tolerable, but for at most 30 seconds. The electrocardiograph was recorded throughout the procedure. The difference between the heart rate before the test and the minimum heart rate during the test was used in the analysis. The profound

bradycardia induced by the test is due to parasympathetic stimulation (60).

5. Cold pressor test.

Sympathetic efferent integrity was assessed by the cold pressor test. Supine subjects immersed one hand to the wrist in melting ice for 2 minutes. Heart rate and blood pressure were recorded in the contralateral arm before and at 1 minute intervals. The results are expressed as the maximum change observed over the 2 minutes.

6. Heart rate variation with deep breathing.

After lying supine on a bed for 10 minutes, volunteers undertook maximum inspiration and expiration for 6 cycles each lasting 10 seconds, with continuous electrocardiograph recording. The variation in heart rate (an increase in inspiration and a decrease in expiration) is due to changes in vagal tone (217). The results are presented as the maximum difference between inspiratory and expiratory heart rate in the final cycle of respiration.

7. Dynamic exercise test.

Dynamic exercise performance was assessed by bicycle ergometry at 175 W for 5 minutes. Blood pressure and heart rate were recorded before exercise and during the 5th minute of exercise. The increase in blood pressure and heart rate during exercise was used in data analysis.

Statistical Analysis

All results are expressed as mean ± SD. Data obtained from cardiovascular measurements were evaluated by repeated measures analysis of variance. The blood pressure and heart rate responses to autonomic tests were compared by paired Student's 't' test (two tailed). The null hypothesis was rejected at $p < 0.05$.

5.3 RESULTS

Tolerance

Perindopril was well tolerated, and no untoward effect was reported spontaneously or on enquiry. No haematological or biochemical abnormality was detected on post study evaluation.

Blood Pressure and Heart Rate

Compared with placebo, perindopril lowered the average blood pressure over the 8 hour study period: the difference in average pressure was 11/7 mmHg in the supine position ($p=0.001/p=0.045$) and was 13/5 mmHg after 1 minute standing ($p=0.001/p=0.006$). The pre-treatment blood pressure was 119 ± 8/64 ± 7 mmHg in the supine position and 121 ± 19/70 ± 9 mmHg standing. The effect on blood pressure was apparent at 1 hour and persisted for at least 8 hours. The fall in blood pressure after perindopril was not associated with a change in heart rate in the supine or standing position (Figures 9 & 10).

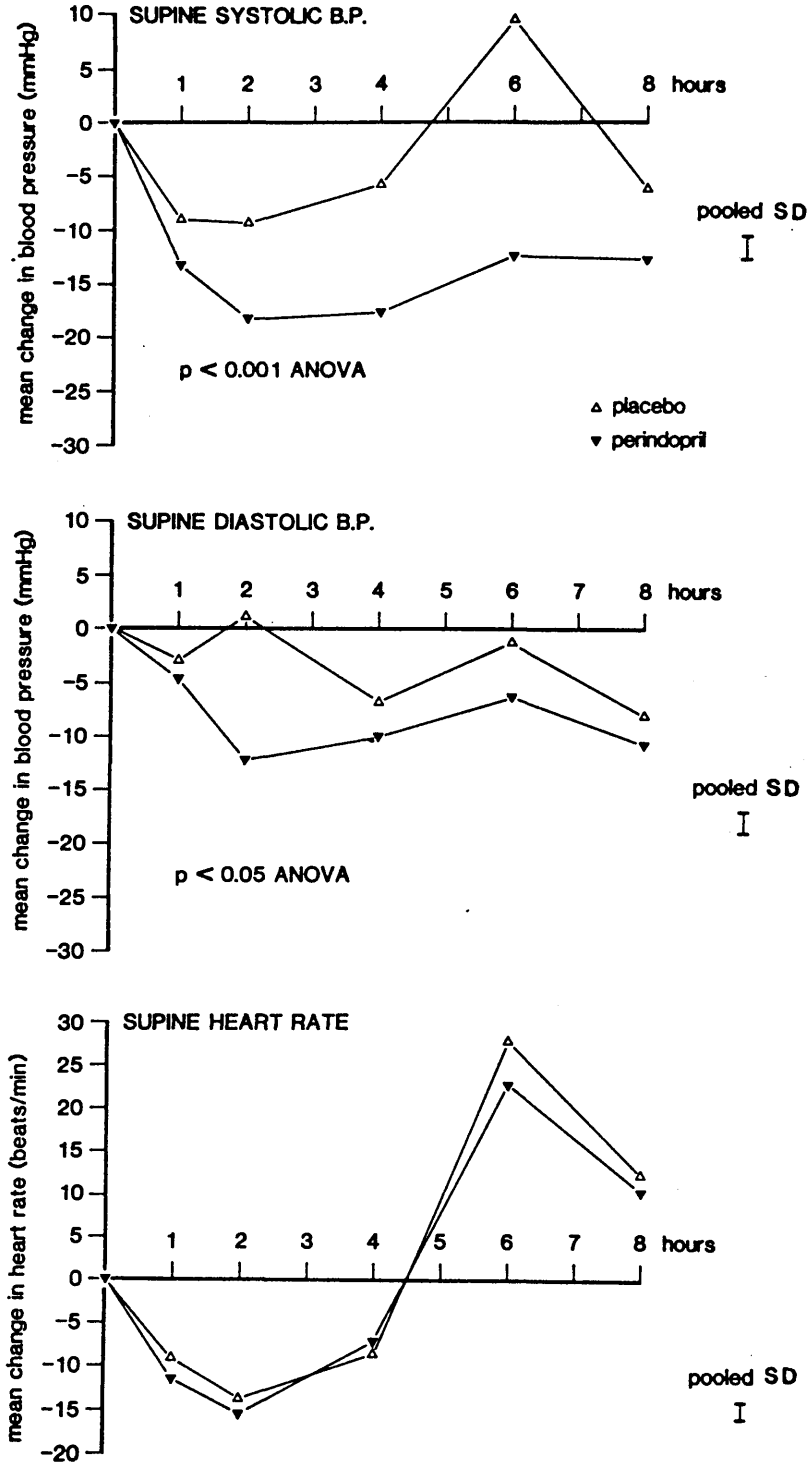


Figure 9. Mean supine blood pressure and heart rate on placebo (△) and perindopril 8 mg (▼) expressed as changes from baseline, n = 10. The fall in pressure was significantly different from placebo by analysis of variance.

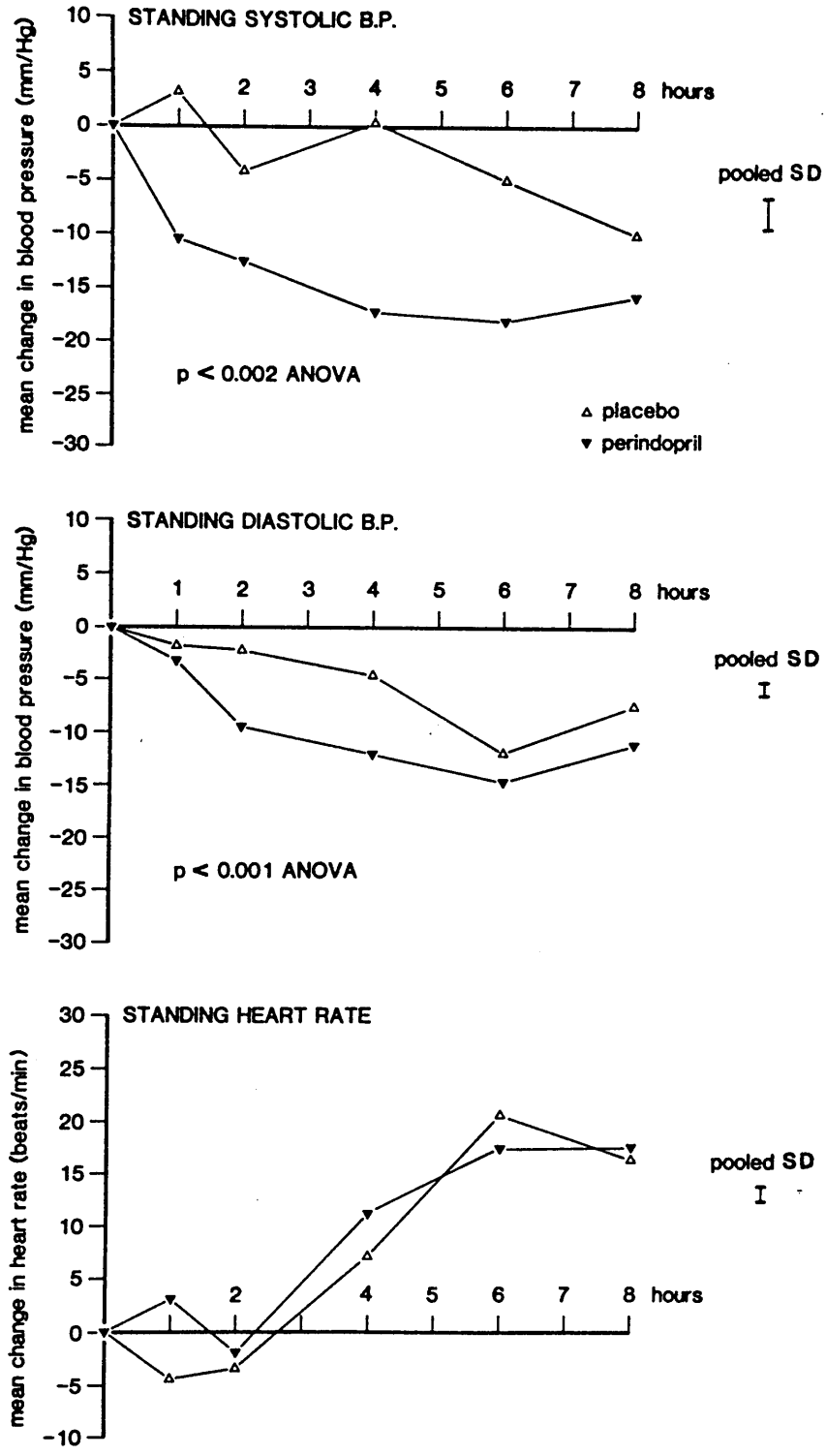


Figure 10. Mean standing blood pressure and heart rate on placebo (△) and perindopril 8 mg (▼) expressed as changes from baseline, n = 10. The fall in pressure was significantly different from placebo by analysis of variance.

Standing to Lying Test, Deep Breathing Test, Diving Reflex

The effect of perindopril on the tests of parasympathetic responsiveness is shown in Table 12. Following placebo, the immediate heart rate response to lying down was a transient increase in heart rate, which was maximal at the 3rd or 4th beat, and thereafter there was a progressive fall in heart rate. Perindopril did not alter the standing to lying ratio although the 95% confidence interval for the paired difference, active - placebo, was -0.3 to 1.3.

On placebo, the maximum heart rate change during deep breathing was 25.7 $\pm$ 7.5 beats per minute. This was increased to 30.4 $\pm$ 6.0 beats.min^{-1} on perindopril ($p < 0.005$). The 95% confidence interval for the difference between perindopril and placebo was 1.6 to 7.8. This result is shown in Figure 11.

Perindopril did not significantly change the bradycardia induced by apnoeic facial immersion in water (the diving reflex).

Isometric Exercise, Dynamic Exercise and Cold Pressor Test

The response to tests of sympathetic function is shown in Table 13. The blood pressure before the procedure tended to be lower on active treatment. Perindopril had no significant effect on the pressor or the chronotropic changes induced by cold stress or by isometric or dynamic exercise. Perindopril did, however, significantly increase the pulse pressure during dynamic exercise: this rose by 10 $\pm$ 20 mmHg from 50 $\pm$ 11 mmHg on placebo, but rose by 36 $\pm$ 19 mmHg from 49 $\pm$ 10 mmHg after perindopril. The 95% confidence interval for the paired difference in pulse pressure increase was 5 to 57 mmHg. Diastolic blood pressure did not rise during dynamic

	Placebo	Perindopril
1. Deep breathing test		
Minimum heart rate ($beats.min^{-1}$)	52.3 ± 10.2	51.5 ± 8.8
Δ heart rate ($beats.min^{-1}$)	25.7 ± 7.5	30.4 ± 6.0**
2. Diving reflex		
Pre test heart rate ($beats.min^{-1}$)	70.3 ± 10.5	73.0 ± 8.9
Δ heart rate ($beats.min^{-1}$)	-26.3 ± 11.8	-26.4 ± 8.5

Table 12. The effect of perindopril on the heart rate variation with deep breathing and the diving reflex (n = 10, mean ± SD, ** $p < 0.005$).

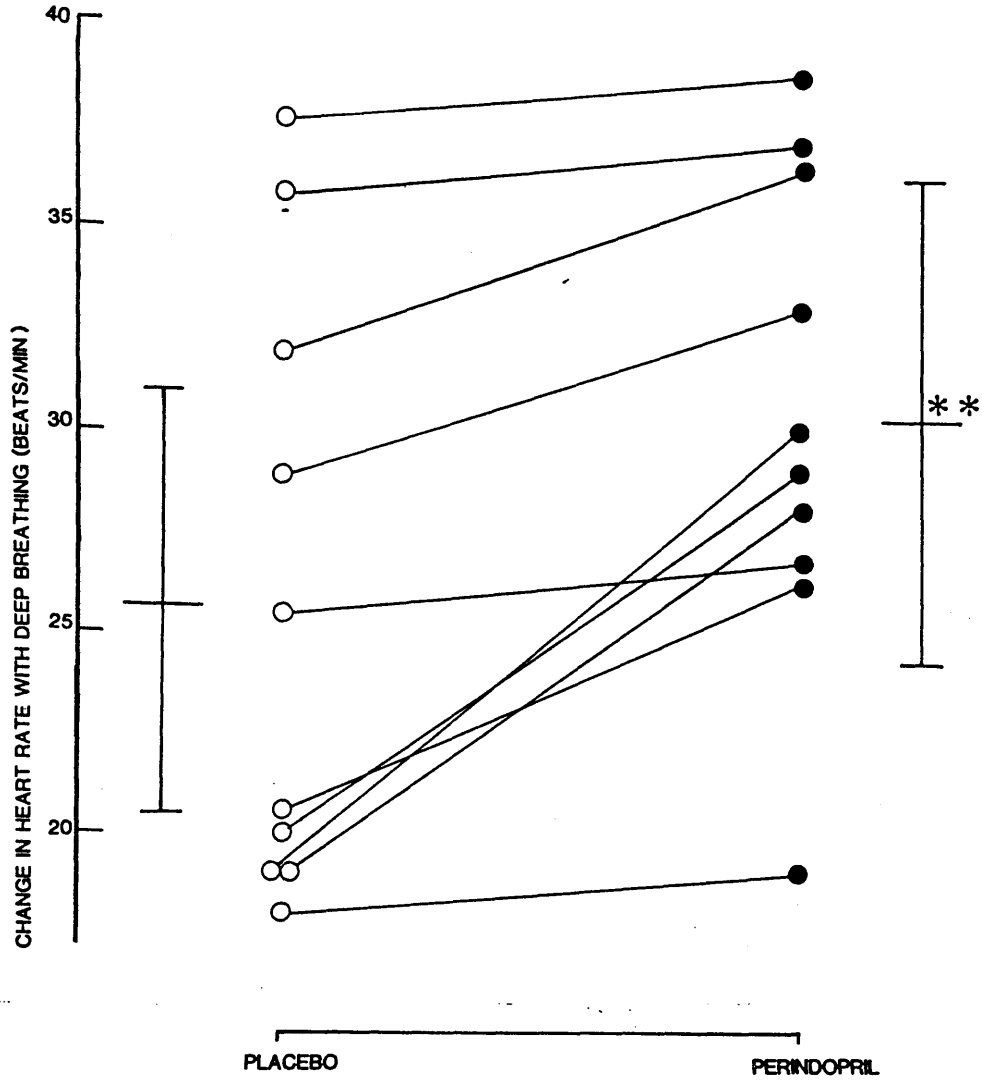


Figure 11. The effect of perindopril on the heart rate variation with deep breathing; ** $p < 0.005$ ($n = 10$, mean $\pm$ SD).

	PLACEBO		PERINDOPRIL	
	Pre test	Δ	Pre test	Δ
Forearm isometric exercise				
Systolic B.P. (mmHg)	119.6 ± 12.4	10.8 ± 8.8	121.1 ± 7.4	15.3 ± 8.2
Diastolic B.P. (mmHg)	60.0 ± 6.4	15.6 ± 9.3	57.5 ± 6.5	10.2 ± 12.3
Heart rate (beats.min^{-1})	58.7 ± 7.0	10.4 ± 9.3	62.8 ± 8.7	7.2 ± 11.4
Dynamic exercise				
Systolic B.P. (mmHg)	124.9 ± 14.7	36.1 ± 19.4	121.1 ± 13.8	45.2 ± 17.3
Diastolic B.P. (mmHg)	74.9 ± 12.4	26.4 ± 2.8	71.7 ± 10.1	8.7 ± 20.1
Heart rate (beats.min^{-1})	70.6 ± 11.0	104.9 ± 11.6	78.7 ± 9.5	9.4 ± 17.7
Cold pressor test				
Systolic B.P. (mmHg)	121.6 ± 13.3	20.5 ± 8.8	118.3 ± 7.0	20.0 ± 11.2
Diastolic B.P. (mmHg)	65.9 ± 8.1	18.5 ± 12.6	64.4 ± 6.1	16.2 ± 13.5
Heart rate (beats.min^{-1})	59.2 ± 8.2	8.6 ± 10.8	60.6 ± 7.6	7.9 ± 8.0

Table 13. The response to exercise performance and the cold pressor test after perindopril or placebo (n = 10, mean ± SD).

exercise after perindopril. Similarly, the pulse pressure increased during isometric exercise on active treatment (+5.1 ± 14.5 mmHg) while it fell as expected on placebo (-7 ± 9.5 mmHg). The 95% confidence interval for the paired difference was 0.5 to 25.1 mmHg.

Valsalva's Manoeuvre

The results of the response to the Valsalva manoeuvre are shown in Table 14. There was no difference in the pressure overshoot or the Valsalva ratio between placebo and perindopril.

5.4 DISCUSSION

These results show that perindopril caused a modest but significant reduction in the blood pressure of salt replete, normotensive volunteers, in keeping with earlier reports of its long-lasting inhibition of the renin angiotensin system (116, Chapters 3 & 4).

The fall in blood pressure was not associated with reflex increases in heart rate, consistent with previous experience with other angiotensin converting enzyme inhibitors (88,143). This observation further confirms that the absence of reflex cardioacceleration is a general property of converting enzyme inhibition.

In the present study with perindopril, a wider array of autonomic function tests has been employed. Perindopril had no effect on cardiovascular changes during the cold pressor test, a reflection of efferent sympathetic integrity (92), and impaired neither isometric nor dynamic exercise performance. The only detectable consequence of

	Pre test systolic B.P. (mmHg)	△ systolic B.P. (mmHg)	Pre test diastolic B.P. (mmHg)	△ diastolic B.P. (mmHg)	Max brad IV (msecs)	Max tach II (msecs)	Valsalva ratio
Placebo	116.8 ± 11.7	5.2 ± 9.0	63.4 ± 7.9	8.6 ± 12.4	1280 ± 213	629 ± 85	2.06 ± 0.34
Perindopril	113.6 ± 6.3	-0.6 ± 7.8	58.3 ± 5.6	4.9 ± 9.3	1206 ± 179	610 ± 92	2.01 ± 0.38

Table 14. Influence of perindopril on Valsalva's manoeuvre (n = 10, mean ± SD).

perindopril was an increase in pulse pressure during exercise, consistent with an arteriolar dilator action following angiotensin II withdrawal. These observations accord with earlier reports with captopril (27,188), acute and chronic enalapril and lisinopril (2,143). Taken together, they suggest that the angiotensin II mediated facilitation of noradrenergic transmission which has been observed in vitro (186,225) may be of little clinical consequence in normal man. Parenthetically, the demonstration of preserved sympathetic responsiveness after angiotensin converting enzyme inhibition does not materially exclude small localised changes in cardiac sympathetic function, nor does it exclude modification of sympathetic function after lesser degrees of stimulation than isometric or dynamic exercise. Indeed, the observation of unchanged plasma noradrenaline levels after angiotensin converting enzyme inhibitors (2,143,188) despite blood pressure reduction, is itself indicative of a "relative blunting" of noradrenergic responses.

The preservation of the responses to the Valsalva manoeuvre and the lack of postural hypotension suggest that baroreceptor reflex function is unimpaired by perindopril.

The enhanced respiratory variation of heart rate with deep breathing, which is under vagal control (217), indicates increased parasympathetic responsiveness after perindopril. Although no statistically significant effect was detected on the immediate heart rate response to lying down, a test of vagal withdrawal (15), the confidence interval for the standing to lying ratio is consistent with enhanced vagal responsiveness. No effect was detected on the diving reflex, a test of maximal parasympathetic stimulation (60),

suggesting that maximal vagal activity is unaltered.

However, the modification of vagal responses which has been reported after captopril (27,188), enalapril and lisinopril (2) and now with perindopril, implies that this autonomic effect is a general property of angiotensin converting enzyme inhibitors probably secondary to angiotensin II removal (3). This vagomimetic action of angiotensin converting enzyme inhibitors, together with an absence of the normal compensatory sympathetic response to hypotension, suggests that the balance between sympathetic and vagal activity is altered during converting enzyme inhibition. Whether vagal enhancement occurs to a similar degree in older or hypertensive subjects is not clear.

Angiotensin II exerts protean effects on autonomic mechanisms including central (119,129,177) and peripheral (166) vagal inhibition, as well as presynaptic facilitation of sympathetic transmission (186,225). In tissues such as the heart, dually innervated by both vagal and sympathetic neurones, the neurotransmitters interact not only by opposing effects on the effector cells, but also by a mutual prejunctional antagonism (128) which may be influenced by angiotensin II withdrawal. Whether the vagotonic effects of angiotensin converting enzyme inhibitors occur at central sites (119,129,177) or peripherally at the heart (166) is not clear. However, the central site of circulating angiotensin II's cardiovascular actions is in the area postrema (110), a zone outside the blood brain barrier (223), and is thus susceptible to changes in circulating angiotensin II. This makes it possible that both central and peripheral sites may be involved.

In conclusion, absence of reflex tachycardia occurs with the angiotensin converting enzyme inhibitor, perindopril. Evidence is provided that enhanced cardiac vagal tone contributes to the cardiovascular effects of perindopril, and that this may be a feature of angiotensin converting enzyme inhibitors in general.

CHAPTER 6

PERINDOPRIL IN HYPERTENSIVE PATIENTS

6.1 INTRODUCTION

Phase I studies with perindopril demonstrated potent and prolonged inhibition of angiotensin converting enzyme and hypotensive activity in salt replete normotensive volunteers (Chapters 3 to 5). The drug was well tolerated in doses of up to 16 mg once daily for periods of up to one week. Hypertensive patients would be expected to show greater sensitivity to the drug, however, and therefore a pilot study was designed to examine the tolerability of perindopril and its biochemical and haemodynamic effects in a small group of patients.

6.2 METHODS

Patients

The inclusion and exclusion criteria for entry to the study are shown in Table 15. The patients underwent full history, physical examination and pre-study tests which included routine urinalysis, haematology, serum biochemistry and an electrocardiogram. Seven patients entered the study and all completed the protocol. Recruitment stopped when a further large scale long term study of the drug was commenced locally. The mean age of the patients was 52 years (range 23 - 65), their mean weight was 76.6 kg (range 59.5 - 105) and height was 167 cm (152 - 176); 2 of the 7 were female.

INCLUSION CRITERIA

Age	18 - 65 years.
Sex	Males or post-menopausal females.
Weight	Within 15% of ideal.
B.P.	145/95 - 200/115 (phase V supine) on two occasions several days apart, after a minimum of two weeks on no antihypertensive drugs.
H.R.	50 - 90 min^{-1}.
Consent	Written informed consent to be granted.

EXCLUSION CRITERIA

Clinically significant disease other than hypertension.

Clinically significant abnormality of pre-study laboratory data, other than electrocardiographic evidence of left ventricular hypertrophy solely.

Known or suspected secondary hypertension.

Asthma, eczema or serious allergic disease.

Medication in the two weeks before the study.

History of drug or alcohol abuse.

Previous exposure to captopril or enalapril.

Table 15. Entry criteria for patients.

Blood pressure at entry was 180 ± 22 / 104 ± 10 mmHg (mean ± SD). Twenty four hour sodium excretion was 155 mmol (range 98 - 216).

Study Design

This was a single blind study. The patients were given a two week course of placebo capsules followed by four weeks of treatment with 4 mg of perindopril daily. After 2 weeks of active treatment, the investigators had the option of increasing the once daily dose to 8 mg if the blood pressure had not fallen from the pretreatment level by 5%.

On the 8th day of the study (during placebo treatment), the 15th day (the first day of active treatment) and the 43rd day (final day of active treatment) the patients attended the Clinical Pharmacology Research Unit at 0830 hours, having fasted since 2200 hours on the previous night. No smoking, caffeine or alcohol ingestion was permitted during the study. An indwelling venous cannula was sited in an antecubital vein and the patients rested until the start of the study.

Blood pressure and heart rate were measured, in duplicate after 10 minutes' supine rest and 5 minutes standing, before and at the following intervals after dosing: 1, 2, 4, 6, 8, 10, 12 and 24 hours. In addition, blood samples were drawn for plasma drug concentration and angiotensin converting enzyme activity at each of these times and samples were collected for plasma renin activity, aldosterone, adrenaline and noradrenaline levels pre-dosing and after 4, 8 and 24 hours.

At 0900 hours, capsules containing the appropriate treatment were administered orally with 200 ml of water. The subjects remained fasting for a further 4 hours and were then given a light standard lunch. Free fluids were permitted from this time. The patients remained seated or supine for the first 4 hours of each study period. The subjects were questioned about any symptoms they experienced at each blood pressure recording time and the pre-study screening tests were repeated after completion of the study.

Laboratory Methods

These have been fully described in Chapter 2.

Statistical Analysis

Repeated measures analysis of covariance was employed to compare with placebo the effect of perindopril on blood pressure and heart rate and on plasma aldosterone, adrenaline and noradrenaline levels. The pretreatment values from the placebo day were used as covariates for the data collected on that day. The pretreatment values from the first day of active treatment, i.e. Day 15, were used for all of the ensuing measurements. Plasma renin activity and angiotensin converting enzyme inhibition were examined by repeated measures analysis of variance. The plasma hormone data were transformed to logarithmic values before analysis in order to normalise the distribution.

Where a significant treatment effect or time-treatment interaction was present ($p < 0.05$), the effects on the three days were then compared with each other at the individual times after dosing by using the 'honestly significant difference' method described by

Tukey (220).

6.3 RESULTS

Tolerance and Safety

All patients tolerated the drug well and none withdrew from the study. There was no evidence of any change in the routine laboratory tests during or after treatment. Four patients were asymptomatic throughout the study; one suffered mild postural dizziness and headache on both placebo and active treatment, one complained of mild chest tightness on one day during active treatment and the final patient experienced mild headache during both active and inactive treatment.

Blood Pressure and Heart Rate

Blood pressure settled slightly but not significantly over the second week of placebo treatment, from 173 $\pm$ 17 / 97 $\pm$ 7 mmHg supine and 177 $\pm$ 19 / 106 $\pm$ 7 mmHg erect to 165 $\pm$ 19 / 95 $\pm$ 7 supine and 168 $\pm$ 16 / 110 $\pm$ 11 mmHg erect (mean $\pm$ SD). Even after taking these values as covariates for the subsequent pressures, there was a fall in pressure after the first dose of perindopril ($p < 0.005$) and this was maintained unchanged after four weeks of active treatment (Table 16). Heart rate did not significantly change. The blood pressure and heart rate profiles are shown in Figure 12.

	Placebo	Acute	Chronic	Pooled SEM	ANCOVA p
SUPINE					
Systolic (mmHg)	163.9	145.2**	142.5**	3.4	0.005
Diastolic (mmHg)	92.7	83.5**	82.1**	1.7	0.002
Heart rate (min^{-1})	68.2	66.5	68.6	1.2	0.464
ERECT					
Systolic (mmHg)	165.3	148.6**	151.3**	2.4	0.001
Diastolic (mmHg)	102.7	92.0**	92.5**	1.4	0.002
Heart rate (min^{-1})	75.7	77.1	81.0	1.6	0.096

Table 16. Blood pressure and heart rate in 7 patients, as mean of readings over 24 hours following dose. ** $p < 0.01$ compared with placebo. There was no significant difference between the acute and chronic days.

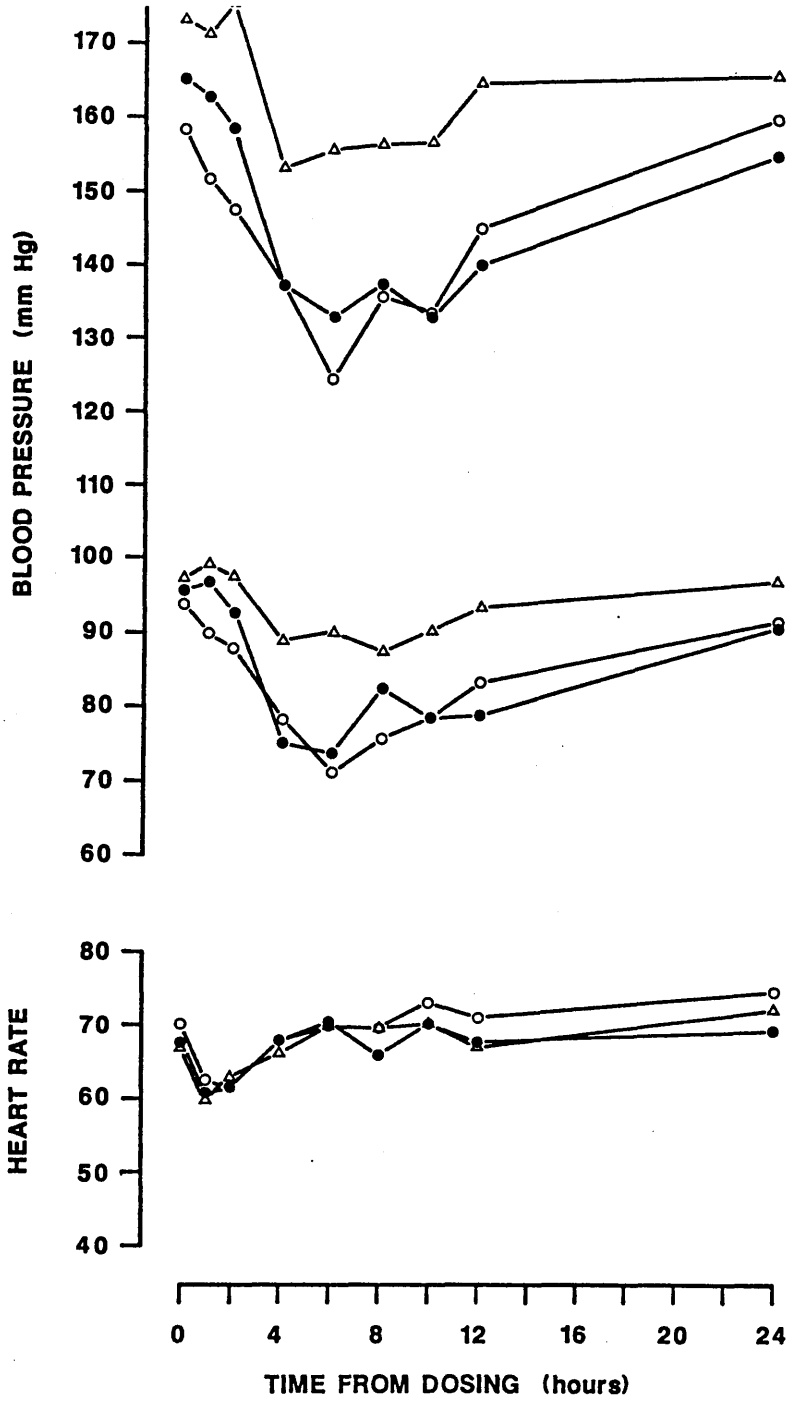


Figure 12. Mean blood pressure and heart rate after placebo (△), perindopril acutely (●) and after one month's treatment (○), n = 7. Blood pressure was significantly lower on both active days than on the placebo day even after correcting for baseline pressure by analysis of covariance ($p < 0.005$).

Angiotensin Converting Enzyme

Inhibition of plasma angiotensin converting enzyme was apparent within 1 hour of the first dose of perindopril and was maintained between 50% and 80% for most of the day. The inhibition 24 hours after the first dose was 49% $\pm$ 20 whereas the 24 hour values on the 43rd and 44th days were 39% $\pm$ 27 and 28% $\pm$ 45 respectively. There was no significant difference between the profiles for the acute and chronic days (Figure 13) when compared by repeated measures analysis of variance.

Plasma Renin Activity and Aldosterone

Perindopril caused an increase in plasma renin activity ($p < 0.005$) but had no detectable effect on plasma aldosterone levels ($p = 0.21$). The median values and 95% confidence intervals are displayed in Table 17.

Plasma Catecholamines

No change was detected in plasma adrenaline or noradrenaline levels during treatment with perindopril. The data are shown in Table 18.

6.4 DISCUSSION

The primary aim of this study was to establish whether the effects of perindopril which were seen in normal volunteers (Chapter 3) would be confirmed in hypertensive patients.

The excellent tolerability which had been reported in the normal subjects was also found in this small group of patients. Even with

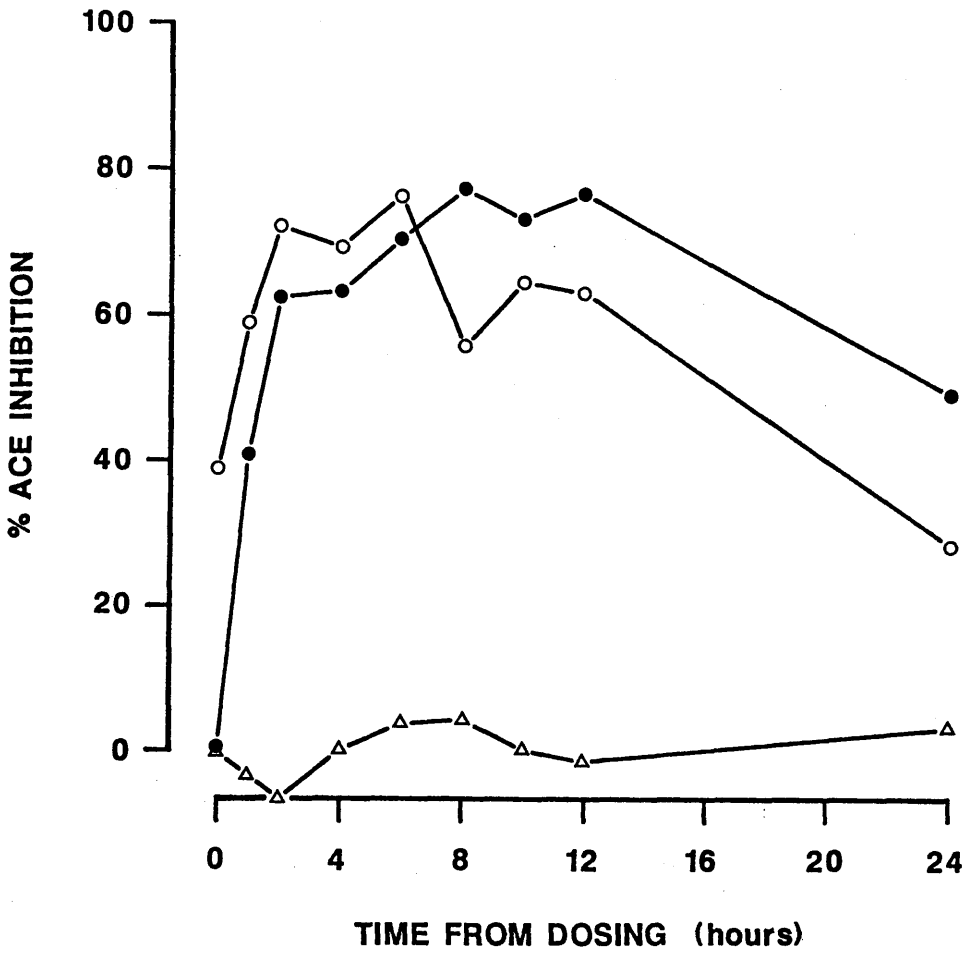


Figure 13. Mean percentage inhibition of plasma angiotensin converting enzyme after placebo (△), perindopril acutely (●) and after one month's treatment (○), n = 7. The acute and chronic profiles did not differ significantly.

		PLASMA RENIN ACTIVITY $ngAI.ml^{-1}.hr^{-1}$		PLASMA ALDOSTERONE $pg.ml^{-1}$	
	hours	median	95% limits	median	95% limits
Placebo	0	1.1	0.5 - 2.2	70	39 - 126
	4	1.2	0.7 - 2.2	42	23 - 76
	8	0.8	0.4 - 1.6	54	33 - 86
	24	0.9	1.6 - 1.3	63	38 - 104
Acute	0	1.1	0.7 - 1.8	73	48 - 112
	4	1.3	0.5 - 3.2	38	18 - 79
	8	1.6	1.0 - 2.4	52	32 - 85
	24	1.6	0.8 - 3.0	54	35 - 86
Chronic	0	2.1	0.8 - 5.7	67	50 - 90
	4	2.4	0.8 - 7.9	36	24 - 53
	8	2.7	0.8 - 8.6	40	26 - 61
	24	2.6	1.3 - 5.4	59	37 - 95

Table 17. Plasma renin activity and aldosterone concentrations after perindopril in hypertensive patients, n = 7. The data were transformed to logarithms prior to analysis. Plasma renin was altered by treatment ($p < 0.005$) but aldosterone did not change ($p = 0.21$). Normal ranges for renin and aldosterone are 4 - 12 $ngAI.ml^{-1}.hr^{-1}$ and 12 - 125 $pg.ml^{-1}$ respectively.

		PLASMA NORADRENALINE nM		PLASMA ADRENALINE nM	
	hours	median	95% limits	median	95% limits
Placebo	0	0.8	0.2 - 3.0	1.0	0.5 - 2.2
	4	0.9	0.4 - 2.2	0.5	0.3 - 1.0
	8	1.4	0.5 - 3.8	0.9	0.4 - 1.8
	24	1.3	0.6 - 3.2	0.8	0.4 - 1.6
Acute	0	1.7	0.5 - 5.2	0.9	0.3 - 2.5
	4	1.7	0.7 - 4.2	0.9	1.4 - 0.9
	8	1.1	0.4 - 2.9	0.7	0.3 - 1.7
	24	1.3	0.4 - 4.6	0.6	0.2 - 1.7
Chronic	0	1.0	0.4 - 2.6	0.8	0.5 - 1.3
	4	1.1	0.5 - 2.6	0.6	0.3 - 1.3
	8	0.7	0.2 - 2.0	0.7	0.3 - 1.4
	24	0.8	0.3 - 2.0	0.8	0.3 - 1.9

Table 18. Plasma catecholamine levels after perindopril in hypertensive patients, n = 7. The data were transformed to logarithms before analysis. There was no effect on either noradrenaline ($p = 0.22$) or adrenaline ($p = 0.97$). Normal ranges are 0.3 - 7.5 nM and 0.0 - 1.0 nM for noradrenaline and adrenaline respectively.

the longer exposure time of one month, no adverse effect was encountered which could be attributed to the drug.

The blood pressure lowering action of perindopril in normotensive volunteers was modest. This study in hypertensive patients was not designed to provide detailed assessment of the effect of the drug on blood pressure. Nevertheless, the blood pressure clearly fell after the first dose of perindopril compared to placebo, even when the slightly lower pretreatment pressure was taken into account. The effect on blood pressure persisted throughout the month of treatment. No symptomatic hypotension occurred. Thus the effect on blood pressure was predictable and useful, even in salt replete patients. A significantly greater hypotensive effect would be anticipated if salt restriction or a diuretic were added (33,50,116,131). In accord with the studies in normal volunteers with perindopril at similar doses (Chapters 3, 4 & 5), with enalapril (143) and with captopril (27,188), no change in heart rate occurred. This is clearly a useful property for a vasodilator drug.

The degree of inhibition of plasma angiotensin converting enzyme which was achieved in the patients was comparable to that encountered in the normal volunteers. There was a trend towards reduced inhibition after four weeks' treatment, however. This has been partially masked by the increase in dose of perindopril taken by some of the patients. Failure to detect inhibition of plasma angiotensin converting enzyme during chronic captopril treatment has been reported (155) but was almost certainly a spurious result due to the rapid decay of captopril in plasma *in vitro* (172).

Despite this slight reduction in measured inhibition of angiotensin converting enzyme, plasma renin activity was still elevated after chronic treatment with perindopril. The median renin activity for the group was still at the lower end of the normal range for our laboratory, in contrast to the effect in normal volunteers (Chapter 3). The patients had considerably lower pretreatment renin activity, however, and their ages ranged up to 65 years. Both factors would tend to reduce the renin activity after treatment (4,213).

In common with the normal volunteers, the patients did not show any response of plasma aldosterone to treatment with perindopril. Once again this may be as a result of several factors: the variability in the data and the small size of the study, the infrequent sampling for aldosterone or a minimal effect on the hormone levels. It remains clear, however, that significant reduction of plasma aldosterone levels is not necessary to achieve the hypotensive effect (211).

No stimulation of the sympathetic nervous system was detected in the patients, despite the relatively greater falls in blood pressure compared to the normal volunteers. Similar findings have been reported for captopril (211) and enalapril (143).

In conclusion, the results of this pilot study of the use of perindopril confirm that the biochemical and haemodynamic effects which were observed in normal volunteers are reproduced in hypertensive patients. Thus perindopril appears to be a safe, well tolerated and effective antihypertensive drug. The results justify proceeding to more extensive testing in patients.

CHAPTER 7

PHARMACOKINETICS OF S-9780

7.1 INTRODUCTION

S-9780 is the active diacid metabolite of perindopril, which is liberated by the *in vivo* ester hydrolysis of the latter drug after oral administration. No data on the pharmacokinetics of S-9780 in man have been published. Studies in animals using radiolabelled S-9780 suggest that it follows triphasic disposition, with a long terminal phase (Institut de Recherches Internationales Servier: personal communication).

The present study examines the pharmacokinetics of S-9780 in healthy volunteers after oral administration of perindopril and after direct intravenous infusion of the active metabolite, S-9780. In particular, the aims were to establish whether the pharmacokinetics of S-9780 in man after intravenous administration were similar to the animal data, to estimate the terminal half-life of the drug and to test whether that half-life was relevant to the prediction of steady state concentrations. From the data after repeated oral dosing with perindopril, it was intended to test for linearity of the pharmacokinetics and to provide information which could be used to examine the concentration-effect relationship (Chapter 8).

7.2 METHODS

Full descriptions of the protocols for the dose-ranging studies with oral perindopril and intravenous S-9780 in healthy volunteers were given in Chapters 3 and 4: only an outline is given here.

Oral perindopril was administered once daily for 7 consecutive days to parallel groups of 6 healthy volunteers, with each group receiving a different daily dose. On the days 1 and 7, blood samples were drawn pre-dosing and after 1, 2, 4, 6, 8, 10, 12 and 24 hours. On the intervening days, samples were taken before dosing. Although the study involved dosing with 1 to 16 mg of perindopril, S-9780 concentrations were only assayed for the subjects taking 4, 8 or 16 mg.

The study of the effects of intravenous S-9780 utilised a latin square crossover design such that 3 doses of S-9780 (1, 2 and 4 mg) or placebo were administered to each of 8 healthy volunteers and blood samples were collected at frequent intervals for 48 hours.

At each time when plasma was collected for estimation of angiotensin converting enzyme activity, an aliquot was retained for determination of S-9780 concentration, by the method described in Chapter 2.

Thus, two sets of kinetic data were obtained: nine plasma levels of S-9780 after the first and the seventh doses of oral perindopril giving 'acute' and 'chronic' profiles at three doses, each in six subjects; and seventeen plasma levels following each dose of intravenous S-9780, giving a total of fifty-one samples, in each of 8

subjects.

Kinetic analysis after oral perindopril

Nonlinear ordinary least squares regression analysis (134) was used to fit separately the acute and chronic data from each subject. Two models were tested: a one-compartment model with first order input and a one-compartment model with zero order input (71). The equations for the zero order model are shown with their derivations in the pharmacokinetics section of Appendix 2 (equations 5, 9, 11 & 12). The coefficients of determination (C_D) were examined in order to compare the models since both models used an equal number of parameters. The coefficient of determination is the ratio of the explained variation (total - residual sum of squares, TSSQ - RSSQ) to the total variation (TSSQ), i.e.

$$C_D = \frac{TSSQ - RSSQ}{TSSQ}$$

The model with the higher C_D was considered to give the better fit (150).

For the zero order model, estimates of clearance/bioavailability, volume of distribution/bioavailability and time to maximum concentration (Cl, V_D and t_{max}) were obtained for each subject, for both the acute and chronic data. Paired comparisons of the parameter estimates obtained for the acute and chronic days were made by the Wilcoxon matched pairs test.

The acute and chronic data were then fitted simultaneously for each subject, giving combined estimates of the parameters. Comparison of parameter values between dose levels was made by Kruskal-Wallis one-way analysis of variance of ranks.

Plasma S-9780 concentrations from pre-dose samples on days 1 to 8 were used to estimate the time to reach steady state. An exponential function, $[C] = A.(1 - e^{-\alpha t})$, was fitted to the data, allowing the half-life to reach steady state to be calculated (half-life = $0.693/\alpha$); it was assumed that it takes between five and seven half-lives to reach steady state.

The area under the curve for subjects taking 4 mg daily was calculated from dose/clearance for subsequent comparison with data from the intravenous study at the same dose. Four mg was chosen simply because this was the dose which was common to both studies.

Kinetic analysis after intravenous S-9780

For each subject, the data from the three administered doses were fitted simultaneously by extended least squares nonlinear regression analysis (160,161,180,196) to several models. This method includes weighting as an extra parameter of the model and thus obviates the need for prior assumptions about the variance of the data. Ordinary least squares analysis was rejected for the intravenous data in view of the considerably greater range of drug concentrations which were measured and the complex relationship between the variance of the assay and the drug concentration (Table 1). The adequacy of each model tested was assessed by use of the -2 log likelihood. This makes an allowance for the different weighting schemes which may be

used when comparing models. The difference between the -2 log likelihood values for two models follows the Chi-squared distribution, with degrees of freedom equal to the difference in number of parameters used in the two models (180).

The equations used took the following general form, with one exponential term for each compartment (72):

$$[C] = D.(A.e^{-\alpha t} + B.e^{-\beta t} + C.e^{-\gamma t})$$

where [C] is the plasma concentration of S-9780 in $ng.ml^{-1}$

D is the dose in mg

t is time in hours

A, B and C have the units $ng.ml^{-1}$ per mg dose

α, β and γ have the units hr^{-1}

The area under the curve was calculated for the 4 mg dose from the equation, $AUC = 4.(A/\alpha + B/\beta + C/\gamma)$. Since the molecular weight of S-9780 is less than that of perindopril, the appropriate conversion factor of 1.3 was used when comparing the areas from oral and intravenous data.

7.3 RESULTS

Oral perindopril

The data fitted best the one-compartment model with zero order input. The model comparisons for the subjects taking 16 mg daily are shown in Table 19. This was the dose at which the peak concentrations

	Zero order			First order		
Subj	TSSQ	RSSQ	C_D	TSSQ	RSSQ	C_D
31	1926	104	0.946	1929	405	0.790
32	1935	60	0.969	1957	180	0.908
33	889	8	0.991	918	67	0.927
34	2258	280	0.876	2260	547	0.758
35	1722	31	0.982	1671	137	0.918
36	407	24	0.941	395	17	0.957

Table 19. Model comparison for pharmacokinetics of S-9780 after 16 mg of perindopril orally: total sum of squares (TSSQ), residual sum of squares (RSSQ) and coefficient of determination (C_D). For both models, the number of parameters was 3 and the degrees of freedom were 5.

occurred latest and for which there was greatest reason to test the alternative model. The one-compartment model with zero order input was used to estimate the following results.

The parameter estimates obtained from the separate fits of acute and chronic data are shown in Table 20. The estimates of clearance and volume of distribution were not significantly different after repeated dosing to those after the first dose.

Estimates of the same parameters from the simultaneous fitting of the acute and chronic data are shown in Table 21. Clearance/bioavailability and volume of distribution/bioavailability were not dose dependent although the time to peak concentration (t_{max}) increased with dose. The measured serum concentration v time profile and the fitted curve for a representative subject are shown in Figure 14. The mean area under the curve from 0 to ∞ for subjects given 4 mg of perindopril was 94.5 $\pm$ 15.5 $ng.ml^{-1}.hr$. Time to reach steady state was estimated to be 43 - 60 hours (Figure 15).

Intravenous S-9780

The model comparisons are shown in Table 22. The three-compartment model clearly offered a better fit for all subjects than either of the reduced models. There was no justification for using a four compartment model. The results which follow were obtained using the three-compartment model.

The estimates obtained for the parameters are shown in Table 23. The average half-lives derived from these parameters were 0.2 $\pm$ 0.04 hours for the first phase, 1.24 $\pm$ 0.2 hours for the second phase and

Subj	Dose	Acute data only				Chronic data only			
		Cl	V_D	t_{max}	C_D	Cl	V_D	t_{max}	C_D
	mg	$l.hr^{-1}$	l	hr		$l.hr^{-1}$	l	hr	
9	4	39.8	337	0.10	0.933	31.6	215	1.17	0.979
10	4	77.2	389	0.11	0.910	43.8	274	0.63	0.993
14	4	41.9	222	0.005	0.986	39.3	236	0.001	0.977
15	4	60.3	247	2.03	0.997	35.1	184	1.24	0.981
17	4	36.9	226	0.01	0.942	34.6	268	0.00	0.919
18	4	42.4	296	0.93	0.974	40.5	252	0.22	0.986
19	8	50.2	488	2.47	0.922	50.3	595	1.33	0.969
21	8	21.7	110	2.88	0.932	20.2	92	3.12	0.957
25	8	38.1	104	5.47	0.944	42.8	230	2.03	0.768
26	8	32.2	181	2.87	0.959	30.4	134	2.90	0.988
28	8	64.4	497	1.41	0.962	48.0	555	1.70	0.984
30	8	44.8	406	0.12	0.954	42.3	421	0.95	0.972
31	16	42.4	276	4.00	0.946	49.6	294	3.07	0.979
32	16	41.8	239	3.00	0.969	47.9	309	2.42	0.885
33	16	44.4	377	3.58	0.991	38.7	321	1.19	0.977
34	16	37.7	285	2.19	0.876	45.4	368	2.74	0.852
35	16	43.2	249	3.34	0.982	37.9	271	2.38	0.983
36	16	63.9	559	3.48	0.941	57.0	580	2.19	0.986

Table 20. Parameter estimates from separate fitting of acute (n = 9) and chronic (n = 9) S-9780 data after oral perindopril: clearance/bioavailability (Cl), volume of distribution/bioavailability (V_D), time to peak concentration (t_{max}) and coefficient of determination (C_D).

Combined acute and chronic data

Subj	Dose	Cl	V_D	t_{max}	C_D
	mg	$l.hr^{-1}$	l	hr	
9	4	37.6	308	0.41	0.93
10	4	58.4	369	0.02	0.91
14	4	40.6	229	0.01	0.98
15	4	45.4	238	1.55	0.91
17	4	35.8	240	0.01	0.93
18	4	42.6	278	0.62	0.98
19	8	53.6	507	2.31	0.90
21	8	21.1	102	2.99	0.94
25	8	37.2	200	2.50	0.73
26	8	32.1	162	2.90	0.96
28	8	54.1	537	1.51	0.94
30	8	44.1	422	0.69	0.96
31	16	47.9	274	3.94	0.92
32	16	43.9	260	2.72	0.92
33	16	42.3	331	2.49	0.77
34	16	41.2	321	2.40	0.80
35	16	40.8	265	2.84	0.96
36	16	61.9	583	2.78	0.93

Table 21. Parameter estimates for S-9780 data after oral perindopril; acute and chronic data fitted simultaneously (n = 18): clearance/bioavailability (Cl), volume of distribution/bioavailability (V_D), time to peak concentration (t_{max}) andcoefficient of determination (C_D).

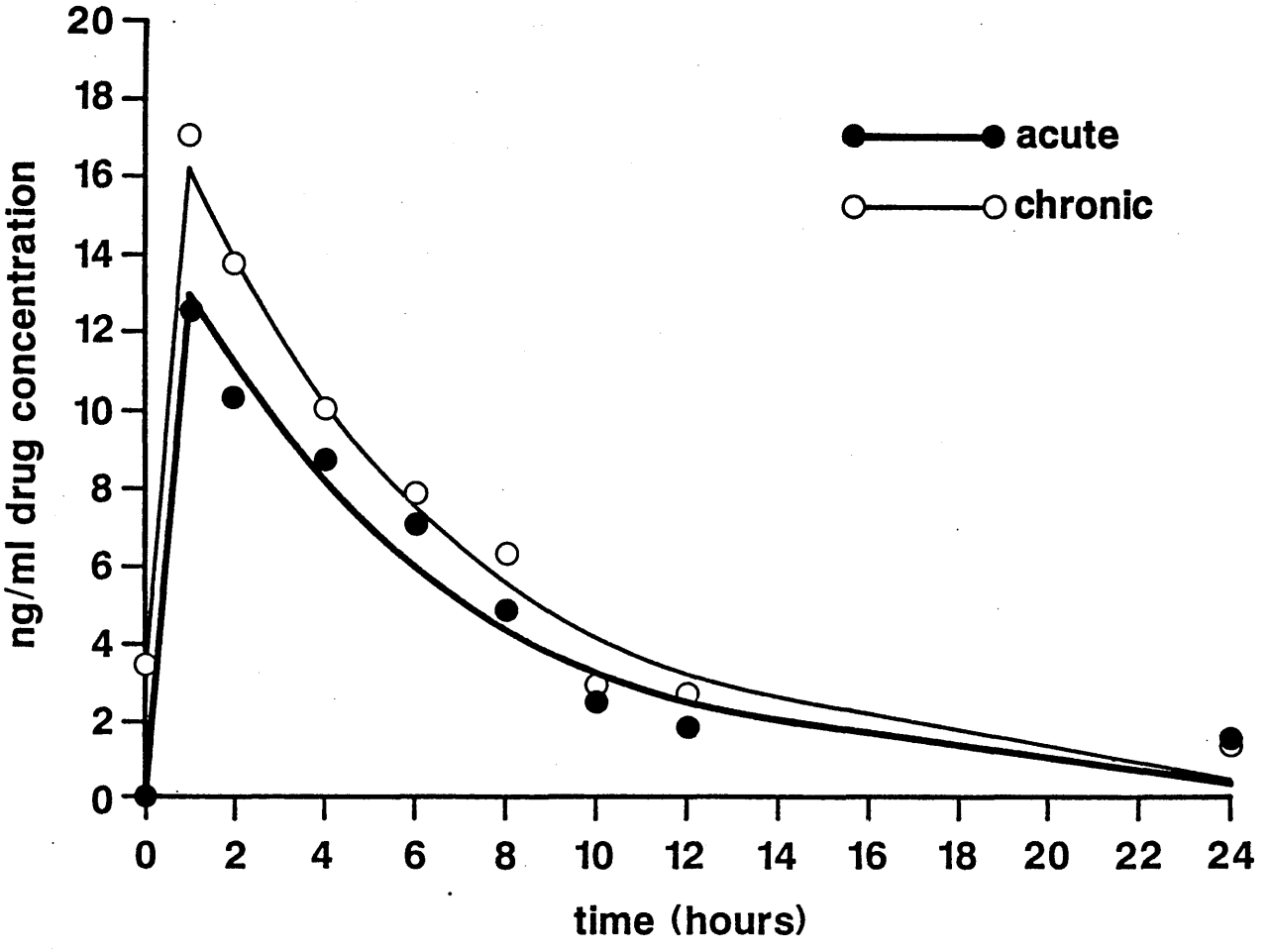


Figure 14. Plasma S-9780 concentrations after the first and seventh doses in a subject treated with perindopril 4 mg orally, showing the fitted curves.

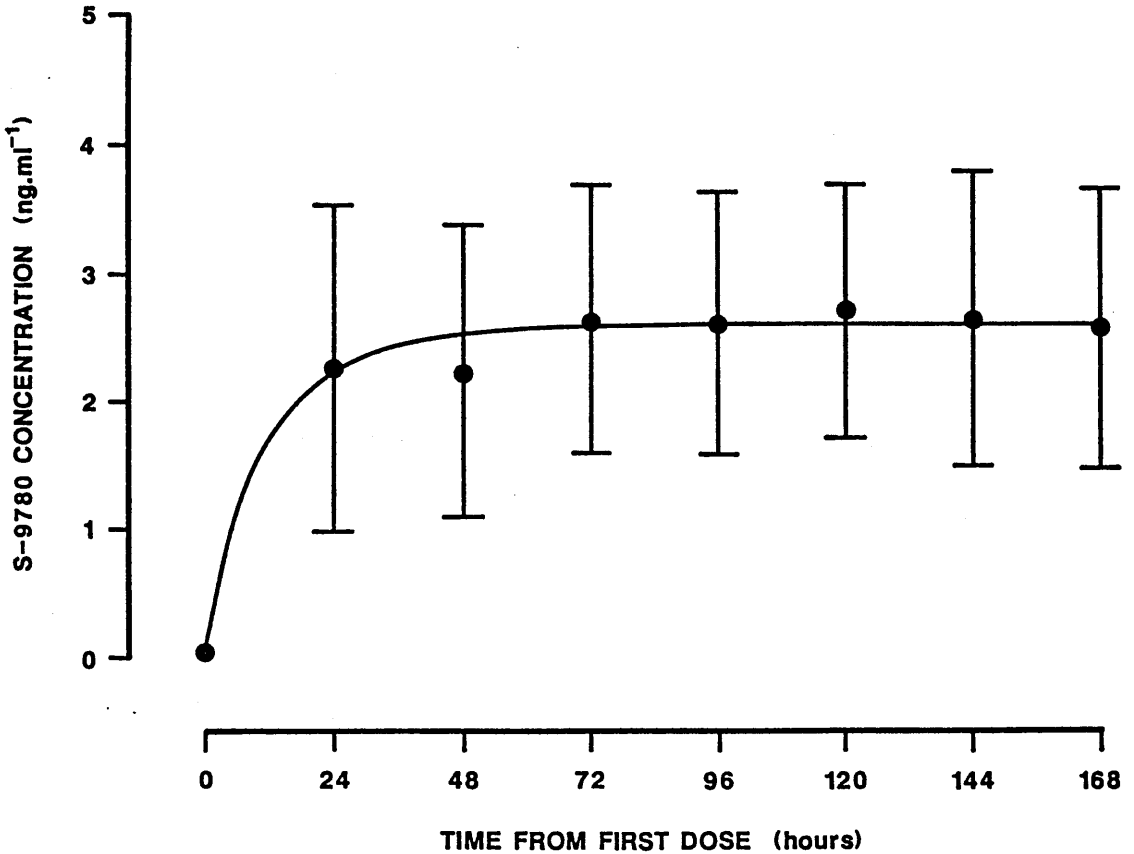


Figure 15. 'Trough' plasma S-9780 concentrations during seven days' treatment with perindopril orally (mean ± SD, n = 18). The curve fitted through the data gives an estimate of the accumulation half-life of 8.6 hours.

Subj	1-compartment -2 log. like.	2-compartment -2 log. like.	3-compartment -2 log. like.	4-compartment -2 log. like.
1	466	442	313 *	313
2	484	434	336 *	336
3	327	301	235 *	235
4	514	464	373 *	373
5	350	317	238 *	235
6	504	448	331 *	331
7	497	447	331 *	331
8	517	517	356 *	356

Table 22. Comparison of models for S-9780 pharmacokinetics after intravenous dosing. As adjacent models differ by two parameters, the critical difference for -2 log. likelihood values at $p < 0.05$ is 6.0; * represents the best model.

Subject	A	α	B	β	C	γ	terminal half-life	C_D
	$ng.ml^{-1}$ per mg	hr^{-1}	$ng.ml^{-1}$ per mg	hr^{-1}	$ng.ml^{-1}$ per mg	hr^{-1}	hr	
1	110	6.2	63	0.70	2.5	0.062	11	.99
2	110	3.1	52	0.49	1.0	0.017	41	.98
3	70	4.1	82	0.70	1.0	0.020	34	.98
4	100	3.3	70	0.51	1.3	0.018	38	.96
5	120	3.2	59	0.51	0.4	0.012	60	.99
6	110	3.0	66	0.47	1.0	0.017	41	.97
7	80	3.9	79	0.68	2.4	0.053	13	.98
8	120	3.0	78	0.54	1.6	0.053	13	.96
mean	100	3.7	68	0.58	1.5	0.040	31	
SD	20	1.1	10	0.10	0.7	0.026	17	

Table 23. Pharmacokinetic parameters derived from intravenous S-9780 data (n = 51) fitted to a 3-compartment model, $[C] = Dose.(A.e^{-\alpha t} + B.e^{-\beta t} + C.e^{-\gamma t})$. C_D represents coefficient of determination.

31.4 ± 17.5 hours for the terminal phase. Data and fitted curves for one subject are shown in Figure 16. The average area under the curve calculated for the 4 mg dose in the eight subjects was 822 ± 125 $ng.ml^{-1}.hr$.

Comparison of the area under the curve after oral perindopril with the area after intravenous S-9780, 94.5 v 822 $ng.ml^{-1}.hr$, gives an estimate of bioavailability for S-9780 of 15%.

7.4 DISCUSSION

The intravenous data were best fitted by a three compartment model This is consistent with the findings after studies with radiolabelled S-9780 in animals (Institut de Recherches Internationales Servier: personal communication). Also in general agreement with those results in animals (Appendix 1) are the estimates of half-lives obtained from this study although this study may have underestimated the terminal half-life: in retrospect, additional samples taken between 8 and 48 hours, and possibly beyond 48 hours, would have been useful. Even so, the terminal half-life is relatively long (over 30 hours) and this figure is not dissimilar to the estimates of 35 to 38 hours which have been quoted for another inhibitor of angiotensin converting enzyme, enalaprilat (94,203).

Despite the apparently slow elimination of enalaprilat, Till et al (197) have demonstrated that steady state enalaprilat levels are

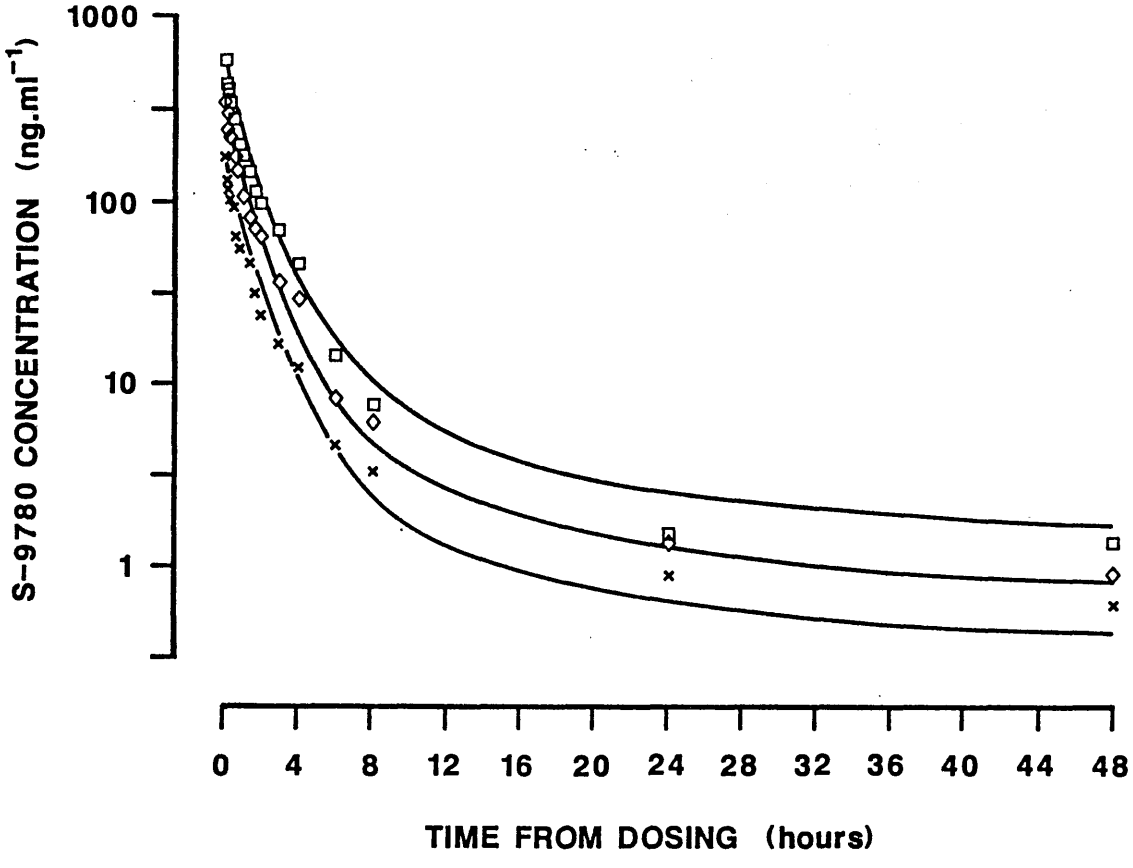


Figure 16. Plasma S-9780 concentrations in one subject following 1 mg (✖), 2 mg (◇) and 4 mg (□) of S-9780 intravenously, with the fitted curves from a three-compartment model.

reached within 30 - 60 hours in normal volunteers. They propose that some form of enalaprilat binding delays excretion of a small pool of drug, but this does not influence excretion of free drug or delay the attainment of steady state. A similar mechanism is likely to exist for S-9780. The data from the perindopril oral dosing study support this hypothesis: steady state appears to be reached after 43 - 60 hours, which is considerably less than the 150 - 210 hours which might be anticipated from a terminal half-life of 31 hours.

The pharmacokinetics of S-9780 do not appear to vary significantly with dose. After oral perindopril 4 - 16 mg, the values obtained for clearance and volume of distribution of S-9780 were equal for each dose. It appears that the pharmacokinetics are linear. Simultaneous fitting of data from the three doses of intravenous S-9780 appears justified. The satisfactory fits obtained also support the method used.

Finally, it is not possible to calculate with certainty the bioavailability of S-9780 from oral and intravenous data obtained in separate groups of subjects. Nevertheless, the age range, sex and weights of the subjects in the two groups did not differ and the clinical and laboratory conditions of the studies were identical. The main source of error in any comparison is likely to be the difference in the kinetic models which were fitted: after oral dosing the one-compartment model will tend to underestimate the tail of the curve. The figure obtained for bioavailability of 15% is unlikely to be widely inaccurate, however, and it provides a useful estimate for the design of future studies involving intravenous administration of S-9780.

The angiotensin converting enzyme inhibition curves which were obtained after perindopril and S-9780 support the assumption that the bioavailability is relatively low, since 1 mg of S-9780 given intravenously produced inhibition of similar degree to 8 and 16 mg of perindopril orally and higher intravenous doses of S-9780 did not cause any further inhibition.

In conclusion, this study has demonstrated that S-9780 data after intravenous administration fit a conventional three-compartment model, giving an estimated terminal half-life of 31 hours. The data from oral dosing with perindopril suggest that bioavailability is relatively low at about 15%, that the pharmacokinetics of S-9780 are linear within the range studied, and that repeated dosing with perindopril for one week does not cause significant changes in S-9780 disposition. Despite the apparent long elimination half-life of S-9780, steady state is reached in healthy volunteers after 43 - 60 hours.

CHAPTER 8

PHARMACODYNAMICS OF S-9780

8.1 INTRODUCTION

For certain drugs, notably theophylline, digoxin and the anticonvulsants, a therapeutic range of plasma concentrations has been defined and can be used in conjunction with plasma concentration measurements in patients to aid in the selection of a suitable dosage regimen for the individual. Where the effect of a drug can be quantified, it may be possible to define the concentration-effect relationship for that drug in an individual subject. If that relationship can be shown to be predictable and to persist after chronic treatment, such information may allow the selection of an appropriate long-term dose when the response to acute treatment is known.

Brunner (26) has suggested that the response of healthy volunteers to inhibitors of angiotensin converting enzyme allows prediction of the therapeutic effects in patients. He has suggested that new angiotensin converting enzyme inhibitors might be developed almost exclusively in normal volunteer studies and that the effects might be extrapolated to patients with the aid of only limited further studies. Such a procedure would probably accelerate the development of new compounds, since the recruitment of suitable patients often appears to be the rate-limiting step in clinical trials.

Experimental work with animals has established the relationship between S-9780 concentration and inhibition of angiotensin converting enzyme *in vitro* (116). The pharmacokinetics of S-9780 in man have been demonstrated in Chapter 7 to be comparable to the pharmacokinetics in animals. If full weight is to be placed on the safety and tolerance information which is available from animal work, then it is also necessary to demonstrate the relevance of the *in vitro* results by studying the relationship between drug levels and effects in man.

This study seeks to determine the relationship between plasma S-9780 concentration and angiotensin converting enzyme inhibition after oral dosing with perindopril and after direct intravenous administration of S-9780 in healthy subjects. The possibility of predicting response at steady state is also addressed.

8.2 METHODS

Details of the protocols, laboratory methods and both the pharmacokinetics and the dynamic results of the studies in healthy volunteers after oral perindopril and intravenous S-9780 have been given in preceding chapters (2, 3, 4 & 7).

Pharmacodynamics after intravenous S-9780

The plasma angiotensin converting enzyme inhibition data from the study of the effects of intravenous S-9780 were related directly to the plasma concentrations of S-9780, measured on the same sample, using the Hill equation (111):

$$\% \text{ ACE inhibition} = \frac{100.\ [C]^{\gamma}}{[C_{50}]^{\gamma} + [C]^{\gamma}}$$

where [C] is plasma S-9780 concentration, $[C_{50}]$ is the concentration of S-9780 which produces 50% inhibition of plasma angiotensin converting enzyme and γ gives a measure of the sigmoidicity of the relationship. Separate fits were obtained for each subject using nonlinear ordinary least squares regression analysis (134). Results are quoted along with the coefficient of determination (C_D), which shows the fraction of the variation in the data which is explained by the model (150).

Pharmacodynamics after oral perindopril

The results obtained for inhibition of plasma angiotensin converting enzyme and plasma S-9780 concentrations after oral treatment with perindopril suggested that the relationship between drug concentration and effect was not simply sigmoidal (Figure 17). It appeared that the response was out of phase with the drug concentration, a situation which can be demonstrated by the occurrence of hysteresis when the response is plotted against drug concentration. This is shown for one subject in Figure 18.

In order to relate the response profile to the drug concentration profile, a model was used which included a term describing the disequilibrium between the two profiles. The pharmacokinetic model from Chapter 7 which described the S-9780 plasma concentrations after oral perindopril was modified for this purpose.

The pharmacokinetic model was itself unchanged but a second compartment relating to the pharmacodynamics was postulated, deriving

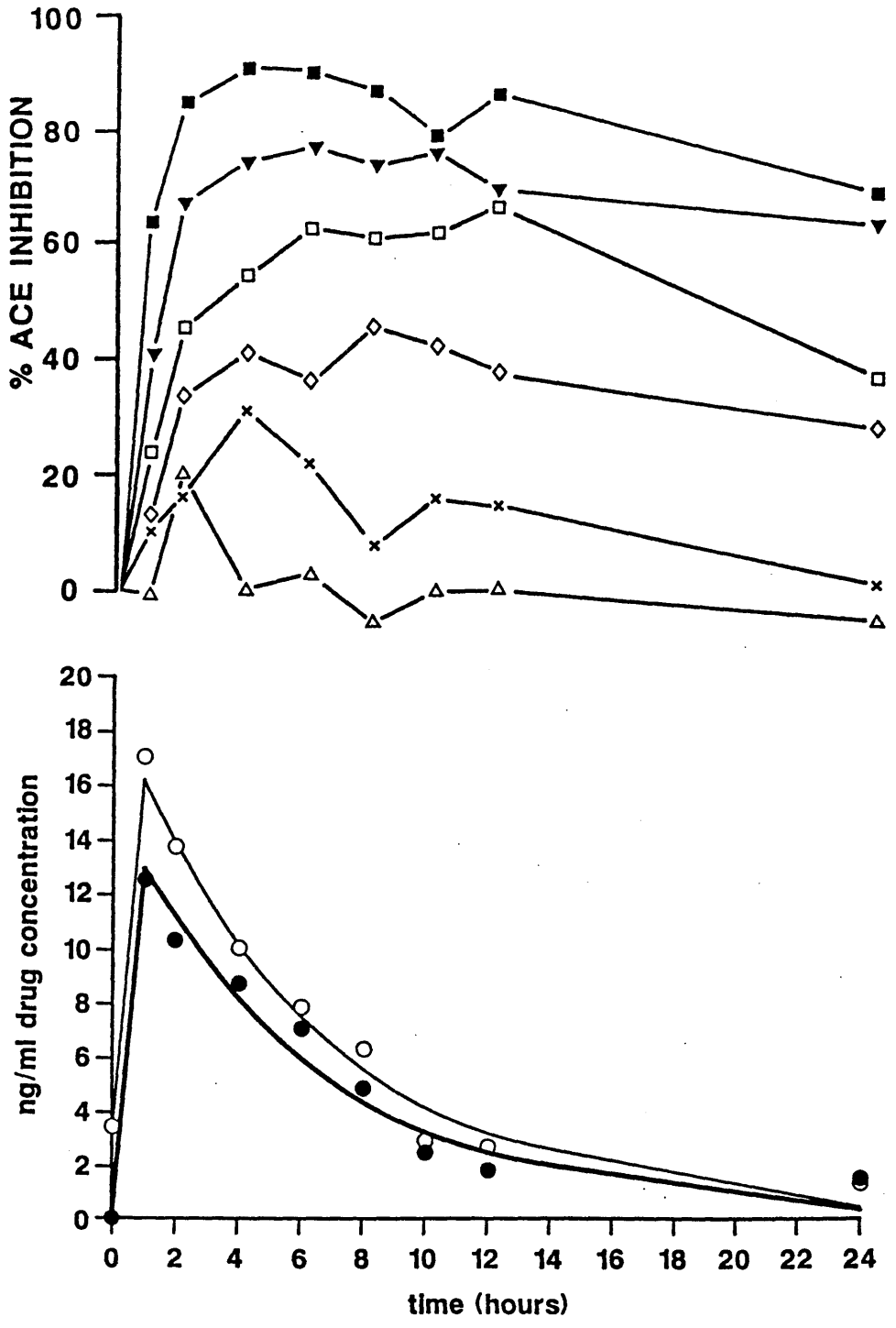


Figure 17. Mean plasma angiotensin converting enzyme inhibition data following oral perindopril, from Figure 4, and plasma S-9780 concentrations in a single subject after 4 mg perindopril orally, from Figure 14: a difference in the profiles is evident.

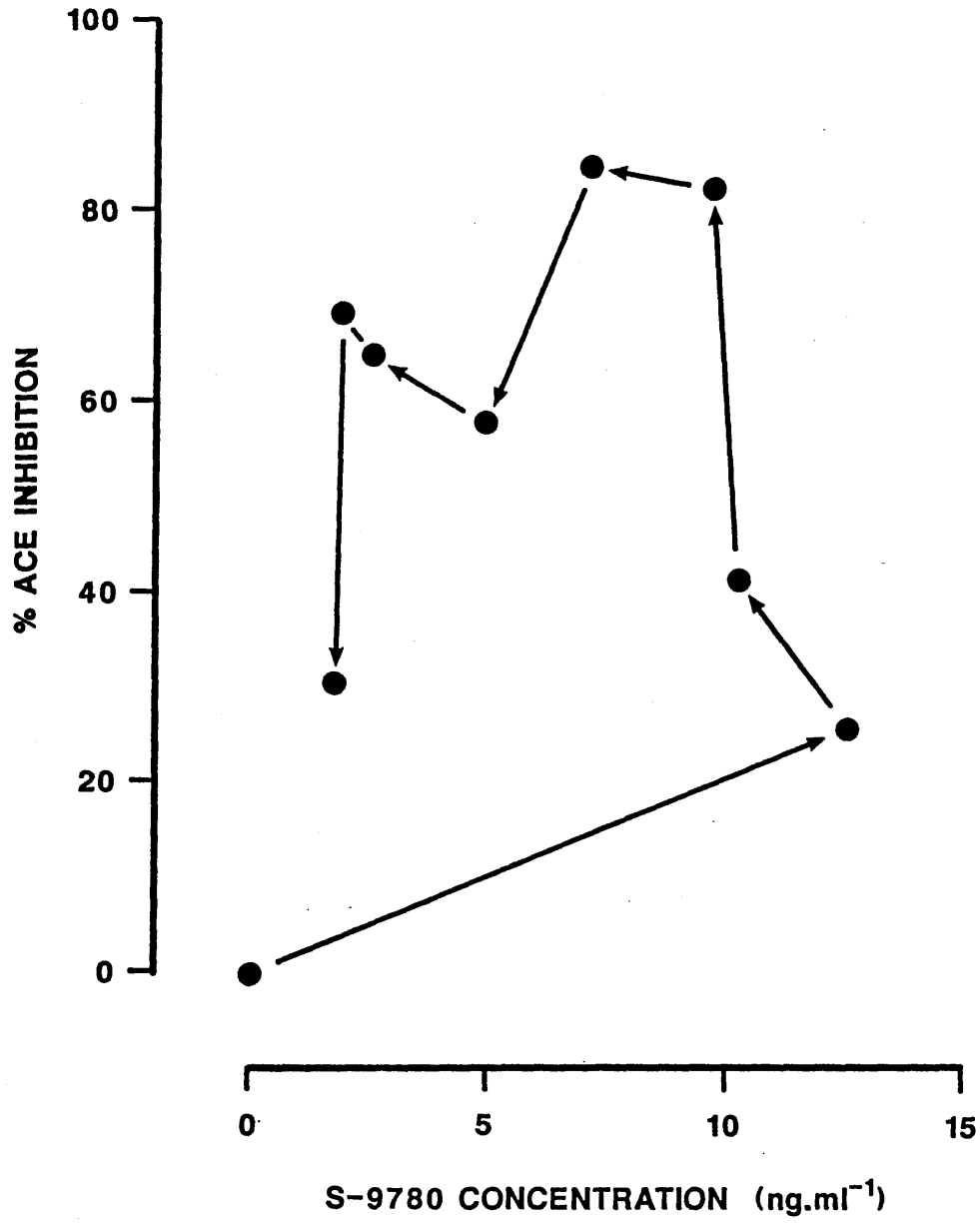


Figure 18. Plasma angiotensin converting enzyme inhibition data plotted against plasma S-9780 concentration in a single subject following perindopril 4 mg orally: a hysteresis effect is demonstrated, confirming that the two profiles are out of phase.

drug from the central compartment by a first order process. Elimination from the second, 'effect', compartment was also governed by first order pharmacokinetics. Such a model has been described by Sheiner et al (181) and is displayed in Figure 19. The concept has been further discussed by Whiting and Kelman (218). The equations which were used and their derivations are given in the pharmacodynamics section of Appendix 2 (equations 15, 17 & 18).

The pharmacokinetic parameters from Chapter 7 were used to predict S-9780 concentrations in the effect compartment, $[C_E]$, at each of the times of angiotensin converting enzyme sampling. The plasma angiotensin converting enzyme inhibition data were then fitted to these concentrations by nonlinear regression analysis (134), once again using the Hill equation. Thus, in this case a further parameter was estimated: keq, the rate constant describing the disequilibrium between drug in the plasma and the effect profiles.

The data from acute and chronic studies were fitted separately using the combined pharmacokinetic values. Reduced models were then investigated by constraining each of the three parameters (keq, $[C_{E50}]$ and γ) in turn to be common to both data sets. Finally, further comparisons were made when two or three parameters were common to both days. For models with equal degrees of freedom, the one which minimised the residual sum of squares was chosen as more appropriate; otherwise, the general linear test (150) was used to compare the models. Comparisons between parameter estimates were made by the Wilcoxon matched pairs test.

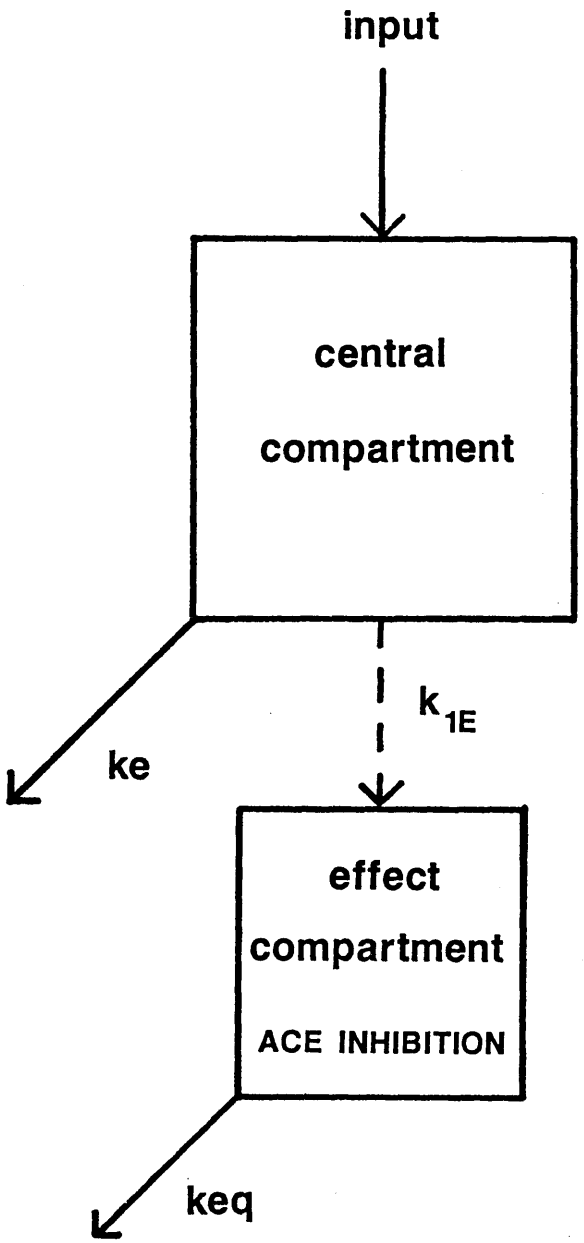


Figure 19. Diagrammatic representation of a one-compartment kinetic model with an 'effect' compartment which does not influence the kinetics but allows for a disequilibrium between plasma concentration of drug and inhibition of angiotensin converting enzyme.

8.3 RESULTS

Intravenous study

The inhibition of plasma angiotensin converting enzyme following intravenous administration of S-9780 was closely related to observed drug concentration, with 74% - 94% of the variation in inhibition being explaind by the drug concentration data. Average values obtained from the Hill equation for γ and $[C_{50}]$ were 0.75 $\pm$ 0.16 and 1.8 $\pm$ 0.9 $ng.ml^{-1}$ respectively. The individual parameters are shown in Table 24 and the data from one subject are shown with the fitted curve in Figure 20.

Oral study

For only two out of the eighteen subjects could the full model, where none of the parameters was common to both data sets, be shown to give a better fit than the model which constrained γ to be common to both days. The latter model was also shown to fit the data as well as or better than any of the other models for the majority of the eighteen subjects. The model comparisons are shown in Table 25.

For all subjects, keq was found to increase after chronic treatment, although in five cases the exact value of the parameter could not be determined due to variation in the data. For the remaining thirteen subjects, the average value of keq increased from 0.22 $\pm$ 0.2 hr^{-1} for the acute dose to 0.63 $\pm$ 0.6 hr^{-1} for the chronic dose ($p < 0.001$). The mean $[C_{E50}]$ for the eighteen subjects increased from 1.6 $\pm$ 1.1 $ng.ml^{-1}$ to 2.7 $\pm$ 2.6 $ng.ml^{-1}$ ($p < 0.01$). The mean value of γ, which was common to both days, was 0.83 $\pm$ 0.46. The individual parameter estimates are shown in

Subject	γ	$[C_{50}]$ ng.ml^{-1}	C_D	degrees of freedom
1	0.92	1.2	0.85	49
2	0.59	0.7	0.81	48
3	0.53	1.1	0.74	32
4	0.85	1.4	0.92	49
5	0.70	3.1	0.82	32
6	0.98	2.9	0.94	48
7	0.74	1.9	0.82	48
8	0.68	2.2	0.91	49
mean	0.75	1.8		
SD	0.16	0.9		

Table 24. Estimates of parameters from the Hill equation after fitting angiotensin converting enzyme inhibition to S-9780 concentrations.

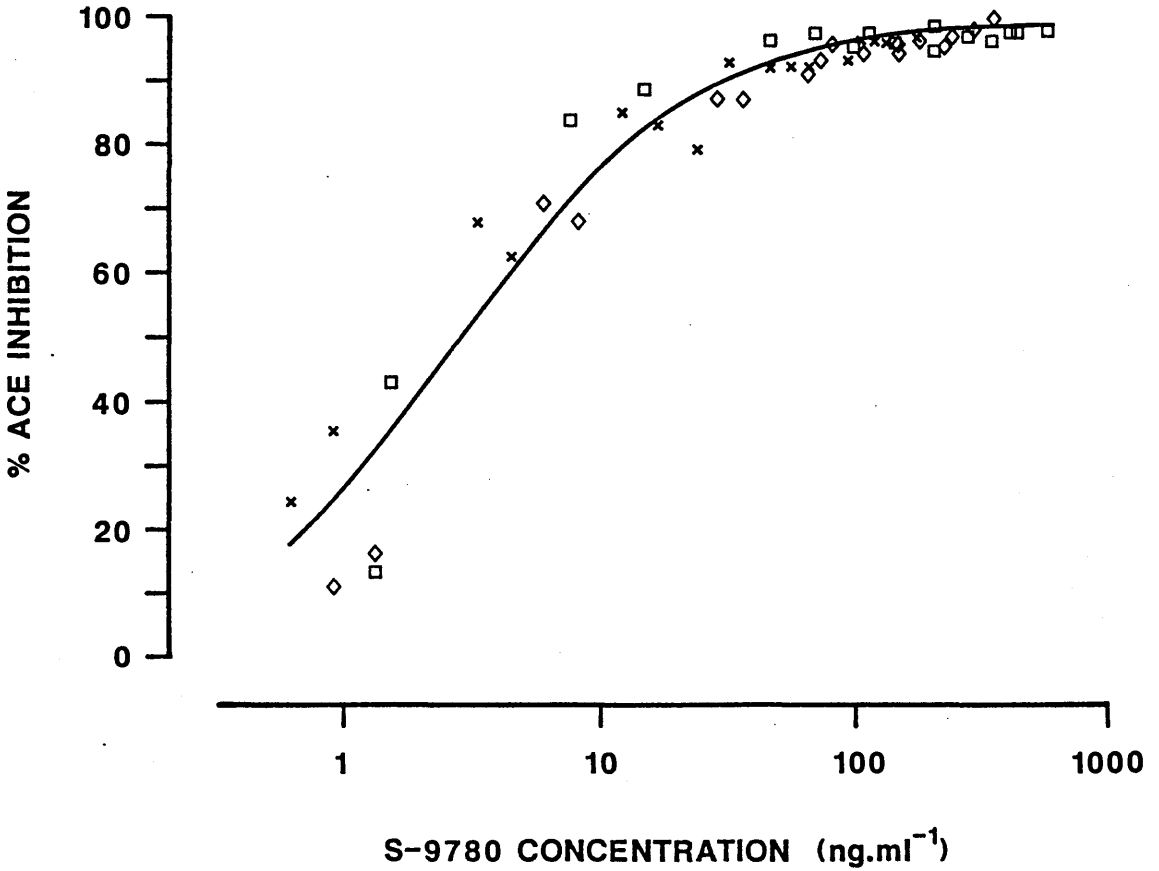


Figure 20. Plasma angiotensin converting enzyme inhibition data related to plasma S-9780 concentrations following acute intravenous administration of S-9780 1 mg (✖), 2 mg (◇) and 4 mg (□). The fitted curve is shown (C_D = 0.94, n = 50).

Subj	Dose	n	Full model		γ common		$[C_{E50}]$ common	
			RSSQ	C_D	RSSQ	C_D	RSSQ	C_D
9	4	18	1053	0.91	1445 *	0.88	2864	0.75
10	4	18	516	0.92	670 *	0.90	690	0.89
14	4	18	710	0.93	853	0.91	735 *	0.92
15	4	16	192	0.96	192 *	0.96	1810	0.62
17	4	18	1670	0.86	1746	0.85	1732 *	0.86
18	4	18	632	0.94	671 *	0.94	679	0.93
19	8	17	722	0.84	876	0.81	772 *	0.91
21	8	18	160	0.98	166 *	0.97	660	0.90
25	8	18	413	0.95	424	0.95	424	0.95
26	8	18	599	0.92	627 *	0.92	685	0.91
28	8	18	163	0.98	510	0.94	227 *	0.97
30	8	18	146	0.98	248	0.96	167 *	0.97
31	16	18	497	0.95	652 *	0.93	701	0.92
32	16	18	254	0.97	267 *	0.97	285	0.96
33	16	18	243	0.96	303 *	0.96	344	0.95
34	16	18	60	0.99	60 *	0.99	71	0.99
35	16	18	410	0.96	446 *	0.96	681	0.94
36	16	18	122	0.98	129 *	0.98	135	0.98

Table 25. Model comparisons for concentration-effect relationship after oral perindopril: residual sum of squares (RSSQ) and coefficient of determination (C_D). The model which appeared to give the best fit is marked by *. The full model had 12 degrees of freedom and the reduced models had 13, thus a higher C_D for the full model does not necessarily imply a better fit: the general linear test was used to test for this (150).

Table 26 and the time course for inhibition from one subject is shown with the fitted curves in Figure 21.

8.4 DISCUSSION

This study has demonstrated that the plasma concentration of S-9780 *in vivo* which produced 50% inhibition of plasma angiotensin converting enzyme was 1.8 ± 0.9 $ng.ml^{-1}$ (mean ± SD). *In vitro* work with guinea pig plasma gave an IC_{50} for S-9780 of 0.82 ± 0.03 $ng.ml^{-1}$ (116). After acute oral dosing with perindopril, the concentration of S-9780 in the 'effect' compartment which produced 50% inhibition of plasma angiotensin converting enzyme was 1.6 ± 1.1 $ng.ml^{-1}$.

There is an apparent disparity between the need to use an 'effect compartment' in order to fit the angiotensin converting enzyme inhibition data to S-9780 concentrations following oral dosing with perindopril and the satisfactory direct correlation which was obtained between plasma S-9780 concentrations and enzyme inhibition following intravenous administration of S-9780. The values obtained for $[C_{50}]$ after acute intravenous dosing and for $[C_{E50}]$ after acute oral dosing are not dissimilar and are particularly low relative to the concentrations of S-9780 achieved after intravenous dosing. Thus, the S-9780 concentrations in the intravenous study produced values for percentage inhibition of angiotensin converting enzyme which lay in a narrow range near the maximum possible. The sensitivity of measuring changes in inhibition within that range is low.

Subj	Dose mg	keq_a hr^{-1}	γ	$[C_{E50}]_a$ $ng.ml^{-1}$	keq_c hr^{-1}	$[C_{E50}]_c$ $ng.ml^{-1}$	$[X_7(0)]$ $ng.ml^{-1}$	C_D
9	4	0.18	1.42	2.9	0.53	7.0	0.0	0.88
10	4	0.23	0.59	1.2	0.25	1.0	0.7	0.90
14	4	0.08	1.72	3.0	0.12	3.4	2.3	0.91
15	4	0.06	1.23	2.3	0.21	9.6	3.0	0.96
17	4	0.06	1.61	2.5	0.23	4.0	1.9	0.85
18	4	0.14	1.42	2.6	0.36	3.9	3.8	0.94
19	8	0.06	0.63	4.0	0.19	5.5	7.4	0.81
21	8	1.66	0.29	1.1	>5	3.1	0.1	0.97
25	8	0.39	0.42	0.7	0.79	0.7	0.1	0.95
26	8	0.26	0.47	0.5	>5	0.9	3.6	0.92
28	8	0.01	0.72	0.1	0.23	0.6	0.8	0.94
30	8	0.08	0.35	0.1	2.20	0.5	2.0	0.96
31	16	0.32	0.73	1.1	0.79	0.5	2.2	0.93
32	16	0.74	0.48	0.6	1.42	0.8	0.9	0.97
33	16	0.42	0.34	0.2	>5	0.4	1.2	0.96
34	16	1.22	0.87	1.3	>5	2.0	5.3	0.99
35	16	0.22	0.91	2.0	>5	3.6	3.2	0.96
36	16	0.56	0.86	1.7	0.85	1.5	2.4	0.98

Table 26. Effect parameters following oral dosing with perindopril, fitting one-compartment pharmacokinetics (combined for acute (a) and chronic (c) days) constraining γ to be common to both days but allowing keq and $[C_{E50}]$ to vary between days; $[X_7(0)]$ represents the drug concentration in the effect compartment at the time of the seventh dose and C_D represents coefficient of determination, n = 18.

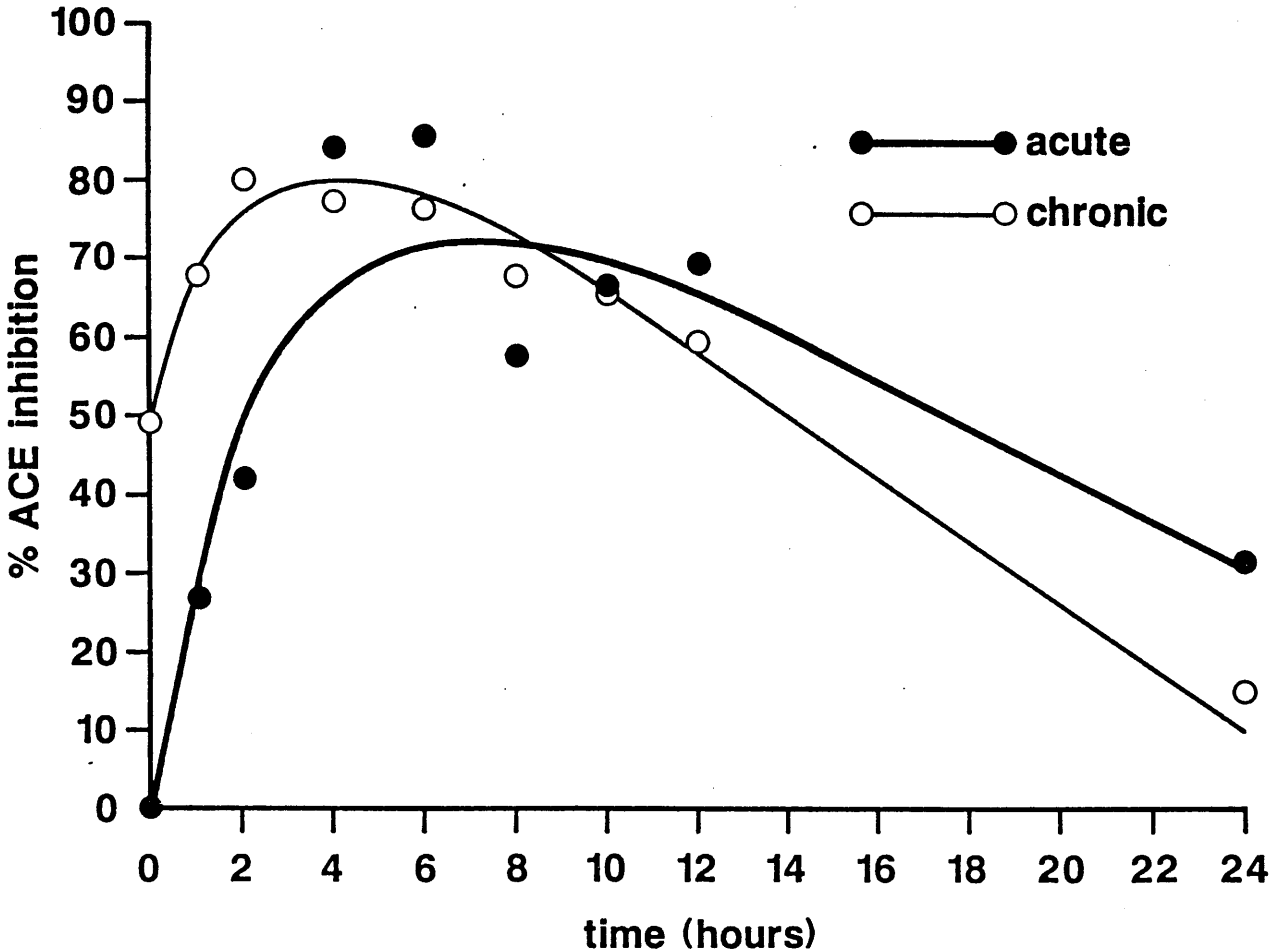


Figure 21. Inhibition of plasma angiotensin converting enzyme related to plasma S-9780 concentration following the first (●) and seventh (○) daily doses of perindopril 4 mg orally in one subject. The fitted curves from the effect model are shown (C_D = 0.94).

As a test of the above explanation, the angiotensin converting enzyme inhibition data from the study of intravenously administered S-9780 were compared with the predicted data from an effect model which used the known intravenous pharmacokinetics along with the parameter estimates obtained from the oral dynamics. The predicted curves were quite dissimilar to the actual data. Thus, if a phase difference between plasma drug concentration and effect also existed after intravenous administration then it should have been apparent under the conditions of the study.

Nevertheless, at the level of plasma concentrations of S-9780 which was achieved after oral dosing with perindopril, there was greater sensitivity in the measurement of inhibition of angiotensin converting enzyme. A clear phase difference existed between the concentration v time profile and the effect v time profile. That difference is described mathematically by the model used. Lack of specificity of the assay for S-9780 would explain such a phase difference: parent drug might have been activated during the early stages of the assay. The careful checks on specificity which were made (Chapter 2, section 5) appear to preclude this explanation.

The value obtained for γ of 0.83 ± 0.46 suggests that the Langmuir equation might have been adequate to fit the data: it represents a special case of the Hill equation, where γ is equal to one. The fuller equation makes no a priori assumption about the sigmoidicity of the relationship.

The purpose of effect modelling is to use data obtained from acute dosing studies to predict the effect which will be achieved at steady

state. The results obtained after oral perindopril in normal volunteers show, however, that the two parameters, $[C_{E50}]$ and keq, increase after repeated dosing. These increases are effectively describing a reduced sensitivity to similar drug concentrations after chronic exposure and more rapid onset and offset of action. No difference in S-9780 pharmacokinetics occurs to explain this observation (Chapter 7). There were insufficient data from this study to establish how long it took for the change in responsiveness to occur. It is not clear whether the alteration occurs in a gradual and progressive manner or whether some qualitative change takes place after the first exposure to the drug. Because of these findings, prediction of the effect at steady state may not be possible from acute studies.

An increase in $[C_{E50}]$ might be accounted for by either of two mechanisms. The binding of drug to a plasma protein to provide an inactive plasma pool may occur, thus causing total plasma drug concentration measurement to overestimate the concentration available for inhibition of angiotensin converting enzyme. The long terminal half-life of the drug (Chapter 7) is consistent with protein binding. The binding protein cannot be plasma angiotensin converting enzyme, however, as suggested by Till et al (197), since no increase in $[C_{E50}]$ should then occur.

The alternative mechanism also provides an explanation for the observed increase in keq. Induction of plasma angiotensin converting enzyme may occur with repeated dosing, such that the expression of the enzyme activity after chronic dosing as a function of the pretreatment values results in spurious underestimates of inhibition.

Unfortunately, expression of the activity in other ways does not help to avoid the problem. Induction of angiotensin converting enzyme has been reported in animals (65) and has been shown to be a possibility in man (63).

If the latter mechanism holds, then a rebound rise in angiotensin converting enzyme activity might be anticipated following withdrawal of the drug. This has not been reported and was not seen in the studies examined here. The slow elimination of the drug may, however, be sufficiently gradual to allow homeostatic mechanisms to curtail excess enzyme production as drug levels fall and to prevent any rebound. Measurement of total angiotensin converting enzyme protein rather than activity would help to answer the question.

The extrapolation of effects on angiotensin converting enzyme to antihypertensive activity remains an area which is fraught with problems. The relationship between the two effects has been shown to be close in some studies (104,170) but other work suggests the converse (211). Blood pressure falls in normotensive subjects tend to be small and the variation large. Satisfactory modelling of blood pressure responses to drug concentration or to inhibition of plasma angiotensin converting enzyme in these subjects is therefore difficult; a similar approach using data from hypertensive patients may prove more productive.

In conclusion, evidence is provided that the plasma concentration of S-9780 which produces 50% inhibition of plasma angiotensin converting enzyme after acute dosing is 1.8 ± 0.9 ng.ml^{-1} (mean $\pm$ SD). Whilst inhibition of plasma angiotensin converting enzyme is

sigmoidally related to plasma drug concentrations after acute intravenous administration, the concentration-effect relationship following oral administration of the parent compound is complex. Repeated dosing with perindopril causes apparently diminished sensitivity of plasma angiotensin converting enzyme to equivalent concentrations of S-9780. This provides further evidence that human plasma angiotensin converting enzyme may be induced by prolonged inhibition.

CHAPTER 9

GENERAL DISCUSSION AND CONCLUSIONS

9.1 DISCUSSION

In the background discussion (Chapter 1) it was suggested that, despite the wide range of antihypertensive agents already available, there was still scope for new agents to be investigated, particularly in classes of drug which were not already over represented. The aims of testing such new drugs were to seek a treatment which was widely tolerated, safe, effective and compatible with established regimes.

The clinical pharmacology of two recently available antihypertensive agents, captopril and enalapril, was discussed. The efficacy of these drugs in hypertension and congestive heart failure is established and there is increasing evidence of their compatability with other treatments. Information concerning the safety of the drugs suggests that they are generally well tolerated but not entirely free from side effects or toxicity. Thus, there was justification for studying a further member of this class of drugs. Initial data on efficacy and safety of perindopril from animal studies (Appendix 1) was encouraging.

Before strict clinical comparisons between perindopril and the established compounds could be undertaken, the tolerability, safety and basic clinical pharmacology of perindopril had to be confirmed in man. The studies described in this thesis set out to determine that information.

It was acknowledged in Chapter 1 that the testing of new compounds in man requires rigorous attention to safety and ethical considerations. It was also accepted that the breadth of information which is required for each new compound is such that multiple studies are needed to collect the necessary data.

In selecting the design of the early studies, a compromise had to be reached. The number of subjects who were exposed to the new compound had to be limited but the maximum information had to be gained and the results had to be sufficiently clear to let the data form the premises for major decisons regarding the design of further investigations.

The dose-ranging study with orally administered perindopril (Chapter 3) provides an example of the successful application of such a compromise. Initially, only two subjects at a time were studied, with low doses of the drug, and were intensively monitored. The hormonal effects were measured as treatment continued and the study could have been terminated at any stage if any untoward effect occurred. The number of subjects studied at each dose was expanded after a higher dose had been found to be tolerated and in this way a profile of activity was obtained at a variety of doses.

As the study progressed it appeared that the likely therapeutic dose of perindopril was about 8 mg. Since an open study is not the ideal design for assessing subjective symptoms or effects on blood pressure, a double-blind, randomised comparison of 8 mg of perindopril and placebo was included.

The detailed information obtained from the study confirmed the activity of perindopril in man, provided evidence of its tolerability and safety after treatment for one week in 30 subjects and suggested a suitable dose for future study.

Studies of the effects of the angiotensin converting enzyme inhibitors on haemodynamics in heart failure are more practically carried out using acute intravenous administration than oral dosing. Since such patients may sometimes be sensitive to small doses of an angiotensin converting enzyme inhibitor, it was necessary to establish the safety and efficacy profile of an intravenous formulation early in the course of the investigations. Once again, a dose-ranging study was chosen but, since repeated dosing was not intended, a double blind crossover design was suitable with dose order determined by reference to a latin square. This provided a study with greater power than a parallel study of similar size.

The discovery that the inhibition of angiotensin converting enzyme was equal with all three doses studied was unexpected. The doses had been chosen in the light of the results of the oral study with perindopril and expected bioavailability of over 50%: the bioavailability of enalaprilat is approximately 60% following oral enalapril administration (203). This information will be particularly valuable in the choice of dose for future studies. There would be no justification for exceeding 1 mg of S-9780 intravenously. As with the oral dose-ranging study, the tolerability and safety profile of S-9780 appeared good.

The oral dose-ranging study with perindopril left unanswered the question of whether perindopril lowered blood pressure in normal volunteers at a dose of 8 mg. It also raised the question of whether perindopril might cause tachycardia, in contrast to the widely reported effects of other inhibitors of angiotensin converting enzyme (88,143,151). A study of the blood pressure response and autonomic effects of perindopril was designed to examine these points. A larger number of subjects was recruited and a double-blind crossover design was employed, with the intention of increasing the power of this study relative to the earlier work.

The questions were answered. Perindopril had effects which were consistent with those of enalapril and captopril on both counts.

Thus, there were grounds for believing that the effects of perindopril on the renin-angiotensin system and the blood pressure were similar to those of captopril and enalapril. Full studies of the safety and efficacy in patients could not be undertaken without evidence of the effects of longer periods of treatment than one week and of the response in hypertensive patients. This group might be expected to show greater sensitivity to the drug than normal volunteers.

A study of one month's treatment with perindopril in hypertensive patients was designed. Since it was wished to limit once again the number of patients exposed to the drug and to gain the knowledge of its effects within a reasonable period of time, the patients were used as their own controls. The statistical analysis took account, as far as is possible, of the inevitable downward trend in blood

pressure over the course of the trial, part of which was unrelated to treatment. The results of the study showed that the drug was well tolerated for longer periods of treatment, that it was effective as an antihypertensive agent and that it appeared to be safe in this group of patients.

The next stage in the investigation was to commence a long-term and larger scale comparison of perindopril with established treatment. This is currently in progress locally, in collaboration with colleagues in general practice.

The dose-ranging studies in normal volunteers permitted the collection of extensive data regarding the pharmacokinetics of S-9780 after direct intravenous administration of S-9780 and afer oral administration of perindopril.

It was reassuring to find that after intravenous dosing the kinetics of the metabolite, S-9780, correspond closely in man to the kinetics in the animals which were used for preclinical toxicological studies. Any difference which was present would tend to diminish the transit time in man.

Of some concern to clinicians was the observation that the terminal half-life of enalaprilat exceeded 35 hours in normal subjects. If that half-life was representative of elimination of drug from the body then accumulation would be anticipated over many days following inception of therapy. A half-life of over 30 hours was also found for perindopril (Chapter 7) but the data from the oral dosing study allowed the accumulation half-life to be estimated at under 9 hours.

This figure is much more consistent with the observed time course of the effect than the higher estimate. It also supports the work of Till et al (197), suggesting accumulation of enalaprilat ceases after 2 to 3 days in normal subjects.

The relationship between the plasma concentration of a drug and its dynamic effect is a field of increasing interest and potential practical application. In Chapter 8, this relationship has been explored for S-9780 after acute intravenous administration, when the effect is clear and easily understood, and after repeated oral administration of perindopril. In the latter case, a fascinating question has been raised but not answered.

It is difficult to explain why a drug which in vitro and, after intravenous infusion, in vivo appears to cause immediate inhibition of plasma angiotensin converting enzyme should produce similar inhibition in vivo following ordinary administration of perindopril only after a significant delay has occurred. It should be noted that drug concentration and angiotensin converting enzyme activity were measured on the same samples. These were stored at -20^{o}C before analysis. There was, therefore, ample time for in vitro equilibration to proceed.

If the assay for S-9780 was not specific to the metabolite then spuriously high levels of drug would be measured soon after dosing which would cause the apparent phase difference. The available information does not support this explanation, particularly as the drug is measured on the basis of its inhibitory activity. An inactive metabolite or pro-drug which was separated from plasma along

with the S-9780 would not register in the angiotensin converting enzyme inhibition stage of the assay.

Conversion of perindopril to S-9780 is promoted by rat plasma. The assay system used rabbit plasma, which does not significantly hydrolyse perindopril, and specificity for S-9780 was confirmed.

If the hysteresis effect had been present for only one or two subjects then it might reasonably have been attributed to biological variation. The effect was consistent for all eighteen subjects after the first dose, however. A change in the concentration-effect relationship was shown after repeated dosing and an explanation for the change is advanced.

Further study of the concentration-effect relationships with perindopril and with other inhibitors of angiotensin converting enzyme should prove interesting in this regard.

9.2 CONCLUSIONS

The main conclusions from the studies described in this thesis are as follows:

1 That perindopril was well tolerated by over fifty subjects treated for periods of up to one month, with no evidence of biochemical or haematological toxicity.

2 That the diacid metabolite of perindopril, S-9780, is a potent and long-acting inhibitor of plasma angiotensin converting enzyme in humans, with predictable and dose-related effects on the renin-angiotensin system.

3 That perindopril 8 mg orally possesses useful blood pressure lowering activity without causing tachycardia or postural hypotension.

4 That perindopril enhances parasympathetic activity and that this action is probably a general feature of the angiotensin converting enzyme inhibitors.

5 That the pharmacokinetics of S-9780 are linear.

6 That the pharmacokinetics of S-9780 following acute intravenous administration of S-9780 follow triphasic decay, with a terminal half-life of 30 hours.

7 That the accumulation half-life of S-9780 after oral

administration of perindopril is under 9 hours and that steady state is achieved in 43-60 hours.

8 That the oral bioavailability of S-9780 is low, probably about 15%.

9 That the concentration of S-9780 which causes 50% inhibition of plasma angiotensin converting enzyme *in vivo* is 1.8 ± 0.9 $ng.ml^{-1}$ (mean ± SD) for man.

10 That the concentration-effect relationship between S-9780 and angiotensin converting enzyme inhibition is complex following oral administration of perindopril but that changes occur after repeated dosing which suggest diminished sensitivity to the drug.

11 That angiotensin converting enzyme may be induced by prolonged inhibition.

12 That studies of a new angiotensin converting enzyme inhibitor in normal volunteers provide useful and relevant information about the likely response in hypertensive patients.

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APPENDIX 1

APPENDIX 1

A1.1 STRUCTURE

Perindopril is (2S,3aS,7aS) 1- [(2S) 1- [(1S) 1-ethoxycarbonyl butylamino] 1-oxopropyl] perhydroindole 2-carboxylic acid terbutylamine salt (Figure 3). It is hydrolysed by esterases *in vivo* to the active diacid form, S-9780.

A1.2 PHARMACOLOGY*

S-9780, the diacid form of perindopril, inhibited guinea pig plasma angiotensin converting enzyme (ACE) by 50% (IC_{50}) at a concentration of 2.4 $\pm$ 0.1 nM (0.82 $ng.ml^{-1}$). A K_i of 1.2 nM was obtained for S-9780 (Dixon Webb plot) with angiotensin I as a substrate. In rabbits, rats, cats, guinea pigs and dogs, S-9780, enalaprilat, perindopril and enalapril decreased, in a dose dependent manner, the pressor response to angiotensin I. The rabbit and the rat were the most sensitive species, with ID_{50} values, respectively, of 2.7 $\pm$ 0.4 and 5.9 $\pm$ 0.3 $\mu g.kg^{-1}$ i.v. for perindopril and 1.2 $\pm$ 0.2 and 2.6 $\pm$ 0.8 $\mu g.kg^{-1}$ i.v. for S-9780.

Perindopril induced a dose-dependent decrease in serum ACE activity in rabbits (0.6 - 20 $\mu g.kg^{-1}$ i.v.) and guinea pigs (10 - 100 $\mu g.kg^{-1}$ i.v.). In conscious rats and dogs perindopril (0.03 - 1 $mg.kg^{-1}$ p.o.) induced a long-lasting inhibition of the angiotensin I-induced pressor response: 40% inhibition was recorded in dogs, 24 hours

after 1 $mg.kg^{-1}$ p.o. Perindopril (0.03 - 0.1 $mg.kg^{-1}$ i.v.) potentiated the increase in femoral blood flow induced by bradykinin injected into the femoral artery of dogs.

In anaesthetised dogs, mean blood pressure and heart rate were not changed after sodium restriction, but the cardiac output was markedly decreased. Perindopril (0.1 - 1 $mg.kg^{-1}$ i.v.) decreased mean blood pressure both in sodium-restricted and sodium-replete pentobarbital-anaesthetised dogs. However, the lowering effect was more pronounced in sodium-restricted dogs.

Perindopril (3 $mg.kg^{-1}$ p.o.) did not change mean blood pressure in conscious dogs maintained on normal-sodium diet but decreased mean blood pressure in conscious sodium restricted dogs.

Plasma renin activity (PRA) and plasma aldosterone concentration were strongly enhanced in conscious dogs maintained on low-sodium diet. Perindopril (3 $mg.kg^{-1}$ p.o.) induced a further increase in PRA associated with a decrease in plasma aldosterone concentration. The degree and duration of ACE inhibition appear to exceed those obtained with enalapril and enalaprilat.

* Summary from published pharmacology by Laubie et al (116) with doses calculated in terms of perindopril base and de-esterified S-9780.

A1.3 PHARMACOKINETICS**

The pharmacokinetic studies were performed in the rat, dog and monkey using single doses of ^{14}C-labelled perindopril (0.5 $mg.kg^{-1}$ i.v. and p.o.). Absorption from the gastrointestinal tract was rapid, with maximum concentrations in plasma after one hour in the rat, dog and monkey. Distribution of radioactivity was wide and was similar for all three species. The fall in radioactivity after intravenous administration was triphasic, giving the following biological half-lives:

	Rat	Dog	Monkey
Half-life	hours	hours	hours
1st phase	0.2 ± 0.1	0.7 ± 0.1	0.5 ± 0.1
2nd phase	1.5 ± 0.6	2.5 ± 0.5	2.1 ± 0.3
3rd phase	67 ± 42	34 ± 4	42 ± 8

A1.4 TOXICOLOGY**

Acute Toxicity

The LD_{50} for oral perindopril in the rat exceeded 3000 $mg.kg^{-1}$ and in the mouse it exceeded 2500 $mg.kg^{-1}$. The signs of toxicity which were seen consisted of diminished motor activity with depressed respiration.

Sub-acute Toxicity

After 5 weeks' treatment with increasing oral doses of perindopril, the minimum lethal oral dose in rats was found to exceed 1000 $mg.kg^{-1}$; the maximum tolerated dose was 30 $mg.kg^{-1}$: above this dose, progressive renal impairment occurred.

In the monkey, the minimum lethal oral dose exceeded 1000 $mg.kg^{-1}$; the maximum tolerated dose was 64 $mg.kg^{-1}$.

Chronic Toxicity

Three months' treatment to rats with 1 $mg.kg^{-1}$ per day was well tolerated, with no evidence of toxicity. Five $mg.kg^{-1}$ per day caused functional renal impairment and 30 $mg.kg^{-1}$ per day led to histological renal damage in 25% of the animals. The equivalent pharmacological doses of enalapril and captopril (60 $mg.kg^{-1}$ and 600 $mg.kg^{-1}$ respectively) caused the same effects but with higher incidences.

Monkeys treated for three months with oral perindopril tolerated doses of 0.5 and 2.5 $mg.kg^{-1}$ per day without effect. Treatment with 10 $mg.kg^{-1}$ per day simply resulted in a reduction of body weight.

Mutagenicity

Tests for gene or chromosome mutations failed to demonstrate any mutagenic activity.

Conclusion

Perindopril may safely be tested in man for periods of up to one month with oral administration.

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APPENDIX 2

APPENDIX 2

A2.1 PHARMACOKINETICS

The data from the oral dosing study were fitted to a one-compartment model assuming zero order input (Chapter 7), using the equations derived as follows.

Let dose input rate, R = dose / t_{max}

and X_1 = amount of drug in the central compartment

Then assuming first order pharmacokinetics,

$$dX_1/dt = R - ke.X_1 \qquad (1)$$

Taking the Laplace transform,

$$s.L(X_1) - X_1(0) = \frac{R}{s} - ke.L(X_1) \qquad (2)$$

First dose

Since for the first dose, $X_1(0) = 0$, equation (2) gives

$$s.L(X_1) + ke.L(X_1) = \frac{R}{s}$$

i.e. $$L(X_1) = \frac{R}{s.(s + ke)} \qquad (3)$$

and by taking the anti-Laplace transform,

$$X_1 = \frac{R}{ke}.(1 - e^{-ke.t}) \qquad (4)$$

Since the concentration in the central compartment ([C]) is X_1 / V_1, where V_1 = apparent volume of the central compartment,

$$[C] = \frac{R}{V_1.ke}.(1 - e^{-ke.t})$$

Since clearance/bioavailability = $V_1.ke$, this can be simplified:

$$[C] = \frac{R}{Cl}.(1 - e^{-ke.t}) \qquad (5)$$

At times when $t > t_{max}$, let $t' = t - t_{max}$:

$$dX_1/dt' = - ke.X_1$$

giving $s.L(X_1) - X_1(0) = - ke.L(X_1)$

$$\Rightarrow \quad L(X_1) = \frac{X_1(0)}{(s + ke)} \qquad (6)$$

and $$X_1 = X_1(0).e^{-ke.t'} \qquad (7)$$

At $t' = 0$, i.e. $t = t_{max}$, $X_1(0)$ is the same as $X_1(t_{max})$ and can be calculated from equation (4) above:

$$X_1(0) = \frac{R}{ke}.(1 - e^{-ke.t_{max}}) \qquad (8)$$

Substitution for $X_1(0)$ from equation (8) into equation (7) gives

$$X_1 = \frac{R}{ke}.(1 - e^{-ke.t_{max}}).e^{-ke.t'}$$

and $$[C] = \frac{R}{Cl}.(1 - e^{-ke.t_{max}}).e^{-ke.t'} \qquad (9)$$

Repeated dosing

After chronic dosing, a further parameter is included representing the amount of drug in the central compartment at the time of dosing, $X_1(7)$.

Thus for $t < t_{max}$

$$dX_1/dt = R - ke.X_1$$

$$\Rightarrow \quad s.L(X_1) - X_1(7) = \frac{R}{s} - ke.L(X_1)$$

$$\Rightarrow \quad s.L(X_1) + ke.L(X_1) = \frac{R}{s} + X_1(7)$$

$$\Rightarrow \quad L(X_1) = \frac{R}{s.(s + ke)} + \frac{X_1(7)}{(s + ke)} \qquad (10)$$

$$\Rightarrow \quad X_1 = \frac{R}{ke} .(1 - e^{-ke.t}) + X_1(7).e^{-ke.t}$$

and $$[C] = \frac{R}{Cl} .(1 - e^{-ke.t}) + [C(7)].e^{-ke.t} \qquad (11)$$

Similarly, for $t > t_{max}$:

$$[C] = \frac{R}{Cl} .(1 - e^{-ke.t_{max}}).e^{-ke.t'} + [C(7)].e^{-ke.t} \qquad (12)$$

A2.2 PHARMACODYNAMICS

It has been postulated that the effect on angiotensin converting enzyme activity may be modelled using the kinetics of the angiotensin converting enzyme inhibitors (17,111). Use of the Hill equation has been suggested (111) since this allows for a maximum response of 100% inhibition and for the 'sigmoid' nature of the dose response curve. There clearly was a time lag between peak drug concentration and peak inhibition of angiotensin converting enzyme in plasma in the case of the oral data (Chapters 3 & 7), however, and therefore a separate 'effect' compartment was postulated. It was assumed that the concentration in this compartment was governed by first order kinetics but that the compartment did not in any way alter the pharmacokinetics previously described (181,218, Figure 19).

i.e.

Let X_E be the amount of drug in the 'effect' compartment and the concentration, $[C_E]$, = X_E/V_E:

$$dX_E/dt = k_{1E}.X_1 - keq.X_E$$

$$\Rightarrow \quad s.L(X_E) - X_E(0) = k_{1E}.L(X_1) - keq.L(X_E)$$

$$\text{giving} \quad L(X_E) = \frac{X_E(0)}{(s + keq)} + \frac{k_{1E}.L(X_1)}{(s + keq)} \qquad (13)$$

First dose

For the first dose, $X_E(0) = 0$. From the kinetics, for $t < t_{max}$ after the first dose, equation (3) was

$$L(X_1) = \frac{R}{s.(s + ke)}$$

Therefore, equation (12) reduces to

$$L(X_E) = \frac{R.k_{1E}}{s.(s + ke).(s + keq)} \qquad (14)$$

giving

$$X_E = k_{1E}.R.[\ \frac{1}{ke.keq} + \frac{1}{ke.(ke - keq)}.e^{-ke.t} - \frac{1}{keq.(ke - keq)}.e^{-keq.t}\ ]$$

and $$[C_E] = \frac{k_{1E}.R}{V_E.ke.keq} + \frac{k_{1E}.R}{V_E.(ke - keq)}.[\ \frac{e^{-ke.t}}{ke} - \frac{e^{-keq.t}}{keq}\ ]$$

Since $k_{1E}/V_E = keq/V_1$, this gives

$$[C_E] = \frac{R}{Cl} + \frac{R}{V_1.(ke - keq)}.[\ \frac{keq.e^{-ke.t}}{ke} - e^{-keq.t}\ ] \qquad (15)$$

For $t > t_{max}$, let $X_E(t_{max}) = X_E$ and $X_1(t_{max}) = X_1$ at $t = t_{max}$:

$t' = t - t_{max}$

$$dX_E/dt' = k_{1E}.X_1 - keq.X_E$$

$$\Rightarrow \quad s.L(X_E) - X_E(t_{max}) = k_{1E}.L(X_1) - keq.L(X_E)$$

From equation (6),

$$\Rightarrow \quad L(X_1) = \frac{X_1(t_{max})}{(s + ke)} \qquad (16)$$

Substitution of equation (16) into equation (15) gives

$$L(X_E) = \frac{X_E(t_{max}).keq}{(s + keq)} + \frac{k_{1E}.X_1(t_{max})}{(s + keq).(s + ke)}$$

$$\text{and} \quad X_E = X_E(t_{max}).e^{-keq.t'} + \frac{k_{1E}.X_1(t_{max})}{(ke - keq)}.(e^{-keq.t'} - e^{-ke.t'})$$

Therefore, since $keq/V_1 = k_{1E}/V_E$,

$$[C_E] = [C_E(t_{max})].e^{-keq.t'} + \frac{keq.[C(t_{max})]}{(ke - keq)}.(e^{-keq.t'} - e^{-ke.t'}) \qquad (17)$$

Repeated dosing

After multiple doses, for $t < t_{max}$, combining equation (10) with equation (13) gives

$$L(X_E) = \frac{X_E(7)}{(s + keq)} + \frac{k_{1E}.X_1(7)}{(s + ke).(s + keq)} + \frac{R.k_{1E}}{s.(s + ke).(s + keq)}$$

$$\Rightarrow \quad X_E = X_E(7).e^{-keq.t} + \frac{k_{1E}.X_1(7)}{(ke - keq)}.(e^{-keq.t} - e^{-ke.t})$$

$$+ k_{1E}.R.[\ \frac{1}{ke.keq} + \frac{1}{ke.(ke - keq)}.e^{-ke.t} - \frac{1}{keq.(ke - keq)}.e^{-keq.t}\ ]$$

and so $$[C_E] = [C_E(7)].e^{-keq.t} + \frac{k_{1E}.X_1(7)}{V_E.(ke - keq)}.(e^{-keq.t} - e^{-ke.t})$$

$$+ \frac{k_{1E}.R}{V_E.ke.keq} + \frac{k_{1E}.R}{V_E.(ke - keq)}.[\frac{e^{-ke.t}}{ke} - \frac{e^{-keq.t}}{keq}]$$

which can be simplified to

$$[C_E] = [C_E(7)].e^{-keq.t} + \frac{keq.[C(7)]}{(ke - keq)}.(e^{-keq.t} - e^{-ke.t})$$

$$+ \frac{R}{Cl} + \frac{R}{V_1.(ke - keq)}.[\ \frac{keq.e^{-ke.t}}{ke} - e^{-keq.t}] \qquad (18)$$

For $t > t_{max}$ after multiple doses, $[C_E(t_{max})]$ and $[C(t_{max})]$ are calculated from equations (18) and (12) and are used in equation (17).

Relationship to ACE inhibition

The drug concentration in the effect compartment, $[C_E]$, was related to percentage inhibition of plasma angiotensin converting enzyme by using the Hill equation:

$$\% \text{ ACE inhibition} = \frac{100.[C_E]^{\gamma}}{[C_{E50}]^{\gamma} + [C_E]^{\gamma}}$$

where $[C_{E50}]$ is the concentration in the effect compartment which produces 50% inhibition of angiotensin converting enzyme and γ gives a measure of the sigmoidicity of the relationship. The conventional term, E_{max}, is replaced in this case by the known maximum inhibition, i.e. 100%.

APPENDIX 3

APPENDIX 3

Some of the work described in this thesis has been presented to the British Pharmacological Society and the Medical Research Society and has been published in the form of abstracts of the Proceedings of the relevant meetings. A list of these is given below.

Lees KR & Reid JL (1984) Single and multiple administration of an angiotensin converting enzyme inhibitor, S-9490-3, in normotensive subjects. British Journal of Clinical Pharmacology, **19 (1)**, 132P - 133P

Lees KR & Reid JL (1985) Effects in normotensive subjects of perindopril, an angiotensin converting enzyme inhibitor. Clinical Science, **69** Supplement 12, 49P

Lees KR & Reid JL (1986) Effects of the angiotensin converting enzyme inhibitor, perindopril, in hypertensive patients. British Journal of Clinical Pharmacology (in press)

Lees KR, Kelman AW, Meredith P & Reid JL (1986) Pharmacokinetics of the angiotensin converting enzyme inhibitor, perindopril, after repeated oral dosing. British Journal of Clinical Pharmacology (in press)

Lees KR, Kelman AW & Reid JL (1986) Effect of repeated dosing on concentration-effect relationships with the angiotensin converting enzyme inhibitor, perindopril. British Journal of Clinical Pharmacology (in press)

The work described in Chapter 4 has been accepted for publication.

Ajayi AA, Lees KR & Reid JL (1986) Effects of angiotensin converting enzyme inhibitor, perindopril, on autonomic reflexes. European Journal of Clinical Pharmacology (in press)