THE HAEMODYNAMIC AND NEUROHORMONAL RESPONSE TO INITIATION OF ANGIOTENSIN CONVERTING ENZYME INHIBITOR THERAPY IN HEART FAILURE

A thesis presented for the degree of Doctor of Medicine to the University of Glasgow, Faculty of Medicine by

97

Iain Boland Squire, BSc (Hons), MB, ChB, MRCP

University of Glasgow
Department of Medicine and Therapeutics
Western Infirmary
Glasgow G11 6NT
Scotland, UK.

1

ProQuest Number: 11007796

ProQuest 11007796

CONTENTS

Index of Figures

9

Chapter 8

Index of tables

Chapter 2

Chapter 3

Chapter 5

Chapter 6

Acknowledgements

The completion of this thesis would not have been possible without the support of Professor John Reid. His advice, friendship and not least patience have been invaluable over the years both with regard to this thesis and in clinical matters. I am also indebted to Dr. Kennedy Lees for his friendship and encouragement over my years in the Department.

The support of numerous medical and nursing colleagues, without whose assistance these clinical studies would have been impossible, is gratefully acknowledged. I am particularly indebted to Dr. W.S.Hillis and Dr. G.T.McInnes for allowing me to approach patients under their care. The continuing friendship of Dr. Richard Donnelly and Dr. Alan Jardine have at different times been sources of great strength.

I am indebted to the outstanding technical asistance of Mr.D.M.Hughes in the assay of plasma ACE activity and drug concentrations. The assistance of Dr. Alison Devlin is gratefully acknowledged with regard to the assay of angiotensin peptides. I owe particular thanks to Dr. Lilian S. Murray for extensive statistical assistance in the analysis described in Chapter 8.

I wish to express my thanks to my parents John and Agnes Squire for all their help and encouragement over many years of study. Their support and love at difficult periods has not been forgotten.

This work is dedicated to my wife Patricia and to our daughter Eilidh Rebecca.

Publications

I.B.Squire, RJ MacFadyen, JL Reid, A Devlin, KR Lees (1996).

Differing early blood pressure and renin-angiotensin system responses to the first dose of angiotensin converting enzyme inhibitors in congestive heart failure. J.Cardiovasc. Pharmacol. 27: 657-666.

I.B.Squire, R.J.MacFadyen, K.R.Lees, J.L.Reid. (1994)

Haemodynamic response and pharmacokinetics after the first dose of quinapril in patients with congestive heart failure. Brit.J.Clin.Pharmac. 38: 117-123

I.B.Squire, I.Violet, M.Chiche, G.Lerebours (1996).

Acceptability of long-term perindopril in the treatment of mild to moderate congestive heart failure. Clin. Cardiol. 19, Suppl.I: I9-I15.

Summary

The studies described in this thesis investigated the haemodynamic response to initiation of therapy with a number of angiotensin converting enzyme inhibitor agents in patients with heart failure. The studies were designed to: (i) define differences among agents in terms of the haemodynamic response to initiation of therapy (ii) investigate possible mechanisms for observed differences among agents in this regard (iii) investigate the relationship between plasma concentration of ACE inhibitor agent and the blood pressure response, and (iv) investigate the relationship between baseline clinical and physiological variables and the blood pressure response.

Differences were observed among ACE inhibitor agents in terms of the blood pressure response to initiation of therapy. While similar blood pressure responses were observed to single oral doses of the ester prodrug ACE inhibitors enalapril and quinapril, the blood pressure response to oral perindopril did not differ from that seen with placebo.

Two possible mechanisms, steric hindrance and "slow, tight binding", for the observed differences were explored using assay of both plasma ACE inhibition and relative concentrations of angiotensin I and angiotensin II. No evidence for either mechanism was gained from these studies.

Using pharmacodynamic/pharmacokinetic modelling a direct linear relationship between the pharmacodynamic effect and plasma drug concentration was derived. Marked interindividual variability was apparent in the blood pressure response to oral initiation of ACE inhibitor therapy. The blood response was more consistent when interindividual pharmacokinetic differences were minimised by intravenous dosing.

No consistent relationship could be identified between any single baseline physiological variable and the blood pressure response. The strongest relationship was with baseline mean

arterial blood pressure. Forward stepwise regression analysis was used to identify the best combinations of predictive variables. The best of these, containing the variables, mean arterial pressure, plasma renin activity, 1/creatinine concentration, age and the ACE inhibitor agent, could explain approximately 25% of the variability in blood pressure response.

In conclusion, differences in the individual blood pressure responses to initiation of ACE inhibitor therapy in CHF appear to be largely dependent upon interindividual pharmacokinetic differences. Accurate prediction of the blood pressure response to initiation of therapy is not possible using readily available laboratory and clinical criteria.

CHAPTER 1

INTRODUCTION

The recognition of the therapeutic benefits of vasodilatation in general and the angiotensin converting enzyme (ACE) inhibitors in particular in congestive heart failure (CHF) represents one of the major medical advances of the second half of the 20th century. The burgeoning costs of health care has over the same period seen the advent of the practice of evidence based medicine, ie the application of the results of large randomised controlled clinical trials to the everyday practice of medicine. In this regard the case for the use of angiotensin converting enzyme inhibitors is established beyond doubt. In spite of the evidence of their benefit in CHF, large numbers of patients who might benefit are currently not prescribed an ACE inhibitor.

A number of case reports and the results of one controlled study have led to physicians concerns of the potential harm they may do by initiating therapy with these agents including first-dose hypotension. However the true extent of the phenomenon and its origins are unclear. The available evidence suggests that the name "first-dose" hypotension is inappropriate and that the blood pressure response to a given agent is consistent within the one individual.

The studies described here explore apparent differences among ACE inhibitor agents in terms of the blood pressure response to initiation of therapy in patients with stable CHF. A series of studies in elderly patients with CHF are described. The possible mechanisms of the inter-agent differences are investigated via comparison of the haemodynamic and neurohormonal profiles to initiation of therapy with a number of agents administered orally or intravenously. The potential use of a specific assay of

circulating levels of angiotensin peptides for the assessment of the level of activity of the renin angiotensin system is explored.

Much has been made of the possible predictive factors of the extent of the blood presure fall in response to ACE inhibition. A systematic analysis of the relationship of a large number of routinely available clinical and laboratory parameters on the one hand, and the blood pressure response on the other, in a population of patients with mild to moderate CHF is described.

CHAPTER 2

THE PATHOPHYSIOLOGY AND THERAPY OF CONGESTIVE
HEART FAILURE

2.1 BACKGROUND

Of all the advances in medical therapy in the latter half of the 20th century, those in

the field of the treatment of heart disease are as remarkable as any. Effective

pharmacological treatments have altered the prognosis for both the patient with

hypertension and the patient with ischaemic heart disease. The prolonged life expectancy

of patients in these categories has however been accompanied by a growing awareness of

the long-term sequelae of both hypertension and ischaemic heart disease, and in particular

the syndrome of congestive heart failure.

Congestive heart failure (CHF) can be considered as a condition in which the heart

fails to fulfil its role as a pump and cardiac output is insufficient to meet the metabolic

needs of the body. The characteristic symptoms of CHF are fatigue, dyspnoea at rest or on

exertion, accompanied by reduced exercise tolerance, and oedema (Poole-Wilson 1988).

The accompanying signs are varied and include peripheral oedema, pulmonary oedema

(detected clinically or radiologically), added heart sounds and cardiomegaly. Thus

"congestive heart failure" is not simply a diagnosis but a constellation of symptoms and

signs, a syndrome attributable to ventricular dysfunction of whatever aetiology. The

relationship of the signs and symptoms of heart failure to underlying disturbances of

central or peripheral perfusion, tissue metabolism and neurohormonal response is not clear

(Editorial 1989). There is no doubt however as to the relentlessly progressive nature of

the condition and its accompanying morbidity and mortality (Schocken et al 1992). Congestive heart failure is an increasingly common condition which affects approximately 1% of the population of Western society. An estimated further 2% of the population have the precursor of overt CHF, namely asymptomatic left ventricular dysfunction. Much of the current interest in CHF stems from the increasing awareness of the prevalence of the condition, and in particular the dramatic improvements in both symptoms and survival which are brought to the condition by vasodilator therapy in general and the angiotensin converting enzyme (ACE) inhibitors in particular.

2.2 THE EPIDEMIOLOGY AND DIAGNOSIS OF HEART FAILURE

2.2.1 Epidemiology

Congestive heart failure is one of the most common conditions leading to hospital admission in industrialised society. Estimates of the overall mortality from the condition are difficult to establish. Accurate diagnosis of the condition is difficult, partly as a result of the relatively non-specific nature of the symptoms, especially in the presence of concomitant pulmonary disease (Wheeldon et al 1993).

The increasing prevalence of CHF is at least partly due to the improvement in treatment of and survival from coronary artery disease and hypertension, and to a lesser extent the decreasing prevalence of valvular heart diseases. In contrast to the figures relating to myocardial infarction, heart failure is a growing cause of morbidity and mortality in most industrialised nations. In the U.S.A. the prevalence of the condition is put at 2.5-3 million cases with approximately 400,000 new cases annually.

The incidence of CHF is age related. In the large scale Framingham Heart Study study 0.3% of subjects were thought to have heart failure at admission. Over 34 years of

follow-up, the prevalence rate was 0.8% at 50-59 years of age compared to 9% for those aged over 80 years (Kannel & Belanger 1991). Under 65 years of age more men than women develop the condition, a pattern which is reversed for those over 75 years of age, in whom the rate has been estimated to be as high as 40 new cases per 1000 population per year (Van De Lisdonk et al 1990). The incidence increases with age: 2.8 % in those over 65 years, 0.06 % in those less than 65 and up to 10% in elderly subjects (Sutton 1990, Parameshwar et al 1992, Wheeldon et al 1993). In urban areas of the U.K. a prevalence in the population as a whole of 0.4-2% has been reported. Studies from Sweden have reported prevalence rates of 2% at age 50 to 13% at 67 years of age (Eriksson et al 1989) and between 11% and 17% in those aged 70-75 years (Landahl et al 1984). Similar figures have been reported from Finland, with a marked predominance of men in the age range 45-74 years (Remes et al 1992).

In keeping with the increased prevalence of the condition, hospitalisation rates for heart failure have increased in a number of industrialised societies. In the U.S.A. in 1990 heart failure was the primary diagnosis at hospital discharge for more than 750,000 admissions (Graves 1992). In the U.K. there are approximately 120,000 hospital admissions per year, representing 5% of all adult medical and geriatric admissions (Sutton 1990, McMurray & Dargie 1992). The hospitalisation rate for heart failure in the U.S.A. rose from 82/100,000 in 1970 to 281/100,000 in 1990 (Ghali et al 1990). The increase in rate was entirely attributable to an increase in those over 55 years of age. Similar trends have been observed in studies in Sweden (Eriksson et al 1991) and the U.K. (Sutton 1990). Most recently, data from the Netherlands has confirmed the increasing rate of hospital admissions for CHF, the total number of patients discharged with a principal diagnosis of heart failure increasing from 7377 in 1980 to 13022 in 1993 (Reitsma et al

1996).

2.2.2. The diagnosis of heart failure

The identification and appropriate treatment of patients with CHF remains

problematic. Difficulties exist with the interpretation of the clinical (Ghali et al 1991) and

radiological (Chakko et al 1991) manifestations of CHF. Many of the symptoms of CHF

may be ascribable to coexisting conditions often present in the largely elderly population

with heart failure. Few studies have attempted to address the value of various signs and

symptoms in the diagnosis of CHF. Table 2.1 shows the relative sensitivity and specificity

of the common signs and symptoms of CHF in predicting the presence of the syndrome, as

found in a series of 1300 patients undergoing cardiac catheterisation. The sensitivity of

both symptoms and physical signs is low and although physical signs have high specificity,

any one sign has low sensitivity (Harlan et al 1977). Thus although the presence of signs is

often diagnostic, their absence is of little value in excluding CHF. In a large primary care

study in the Netherlands, the presence of oedema was predictive of CHF in only 11% of

those aged 65-74 and 27% for those > 75 years (Grundmeijer et al 1996).

In the U.K. the majority of contacts between patients with CHF and medical staff is

with general practitioners (Wheeldon et al 1993). In this setting only half of those patients

prescribed loop diuretics fulfil diagnostic criteria for heart failure (Clarke et al 1994). In

the context of the proven benefits of the angiotensin converting enzyme (ACE) inhibitors

in CHF, these agents are prescribed for less than one in five of the patients correctly

identified as having the condition (Clarke et al 1994). Thus in spite of increasing

awareness of CHF as a major health issue it seems that present investigation and treatment

of the condition is inadequate.

Table 2.1

The predictive value of symptoms and physical signs for the presence of CHF (LVEF < 40%) in 1306 patients undergoing cardiac catheterisation.

	Sensitivity (%)	Specificity (%)	Positive predictive value (%)
Medical history			
Dyspnoea	66	52	23
Orthopnoea	21	81	2
Nocturnal dyspnoea	33	76	26
History of oedema	23	80	22
Physical signs			
Tachycardia	7	99	6
Rales	13	91	27
Oedema	10	93	3
Added heart sound	31	95	61
Elevated JVP	10	97	2
Cardiomegaly (Chest X Ray)	62	67	32

Given the clinical difficulty of clinical diagnosis of CHF in many cases, diagnostic investigations are of paramount importance in the identification of patients with CHF. Electrocardiographic and radiological investigations have an important but limited role in this respect. At the present time there is no serological test or tests for the diagnosis of CHF. The assay of one of a number of recently identified peptide compounds such as B-type natriuretic peptide (Struthers 1993), various atrial natriuretic peptide fragments (Kelly et al 1995) or adrenomedullin (Richards et al 1996) may lead to the development of a screening test for CHF. Until such a test becomes available, echocardiography will continue to have a pivotal role in the identification of the patient with CHF and is at present the most appropriate initial investigation of patients with suspected heart failure. In spite of the fact that approximately three out of four patients with CHF are referred to hospital either acutely or electively, only around one third of these undergo echo-cardiography (Clarke et al 1994). In this context it is of note that the greatest increase in the demand for echocardiography services has been in the area of the assessment of left ventricular function after myocardial infarction (Gupta et al 1995).

2.2.3 The economic importance of heart failure

Heart failure is the cause of approximately 10 % of acute hospital admissions of those over 65 years of age in the U.K. (McMurray & McDevitt 1990). With such numbers and the chronic nature of the condition with relapses requiring intermittent hospitalisation, CHF represents an important drain on health care resources. It is estimated that for each hospital admission there are 14 general practice consultations (Wheeldon et al 1993). The cost of CHF to the National Health Service in the U.K. has been put at approximately £360 million for the year 1990/91, more than 1% of the N.H.S. budget

(McMurray et al 1993). Almost 60% of this total was taken up by the cost of emergency and elective hospital admissions of patients. Similar figures have been reported for other European countries such as the Netherlands (Koopmanschap et al 1992).

As already discussed, CHF is a condition with a poor prognosis characterised by steady progression of disease interspersed with acute exacerbations. It has been estimated that one major determinant of the cost of CHF to the NHS is how and by whom ACE inhibitor therapy is initiated. Hart & McMurray have estimated that ACE inhibition commenced by the General Practitioner without admission of the patient to hospital results in a lifetime cost saving of £11 per patient treated. Initiation in hospital results in an estimated cost per life-year gained of £747 (Hart & McMurray 1993). However this latter figure still compares very favourably with that accrued from other common procedures (Hay et al 1991, Hart et al 1993). At a time when Health Service resources are becoming more scarce, the accurate diagnosis of a condition as prevalent as CHF, and its optimum management, has become a priority for all health care professionals. For the same reasons, prevention of the condition and identification at an early stage are now prime aims and further research into the patho-physiological mechanisms involved in CHF and potential therapeutic strategies are easily justifiable.

2.3 THE AETIOLOGY OF HEART FAILURE

Very few studies of therapy in CHF have stratified predisposing illness, which may have variable prognoses (Packer 1988). The underlying diagnoses from the Framingham Heart Study and the SOLVD registry are shown in Table 2.2.

Initial epidemiological studies identified chronic hypertension as the major predisposing risk-factor for heart failure with over three quarters of the patients in the

Table 2.2

Aetiology of CHF from Framingham and SOLVD registries

Aetiology of CHF	Framingham		SOLVD registry (EF<0.45)	
	Men	Women	Men	Women
IHD	45.8	27.4	74.3	48.4
HT heart disease	76.4	79.1	4.2	18.2
Valve	2.4	3.2	6.4	10.3
Cardiomyopathy	-	-	14.9	20.9
Other	11.9	16.8	0.2	0.2

(1) Causes in the Framingham study not mutually exclusive.
(2) SOLVD registry included patients with ejection fraction (EF) of < 0.45 or who had radiological evidence of CHF.

IHD=Ischaemic heart disease
HT= Hypertensive heart disease
Valve=Valvular heart disease

Framingham population having been diagnosed as suffering from hypertensive heart disease (Kannel 1989). The risk of developing heart failure was related more strongly to systolic than diastolic blood pressure. Other community based studies have found hypertension to be a rather less common cause of CHF (Eriksson et al 1988, Parameshwar et al 1992, Remes et al 1992). Even within the Framingham study (Table 2.2) a significant proportion of this population had intercurrent ischaemic heart disease and the latter is now the most common condition predisposing to chronic CHF in industrialised society.

In the Studies of Left Ventricular Dysfunction (SOLVD) registry, ischaemic heart disease was the main underlying disease, being the predisposing factor to CHF in 74% of patients with an ejection fraction of < 0.45 in comparison to only 4% with hypertensive heart disease (Bangdiwala et al 1992). Data from U.K. studies have yielded broadly similar results (Sutton 1990). Valvular heart disease and cardiomyopathies represent a small proportion of the overall CHF population.

The presence of ischaemic heart disease, in particular a history of myocardial infarction is a powerful risk factor for the development of CHF (Kannel 1989). Hypertension with ECG evidence of left ventricular hypertrophy confers a markedly elevated risk (Kannel et al 1994). Diabetes appears to be a relatively greater risk factor for females (Ho et al 1993a). In the presence of any of the underlying aetiological conditions, the presence of cardiomegaly, left ventricular hypertrophy or diabetes mellitus increases the likelihood of developing heart failure.

2.4 PROGNOSIS

2.4.1 Morbidity and mortality

As noted above the rates of hospitalisation of patients with heart failure are

high. One year after diagnosis, 40% of heart failure patients on the SOLVD registry had been hospitalised at least once. Approximately half of these admissions were for worsening heart failure; a further 25% were for other cardiovascular causes such as myocardial infarction, cardiac surgery, unstable angina, thromboembolic event and arrhythmia; the remainder were for non-cardiovascular reasons. Mortality from heart failure appears to be on the increase, even allowing for the increasing numbers of elderly people in the population. Heart failure was listed as the primary or a contributory factor on 230,000 death certificates in the U.S.A. in 1988, double the number in 1970. The increase in death rates from heart failure has been seen in both sexes (Yusuf et al 1989). In the Framingham study only 25% of men and 38% of women with heart failure were alive 5 years after diagnosis (Ho et al 1993b). Similar figures have been reported from other studies (Eriksson et al 1991). The annual mortality rate among heart failure patients is approximately 10% (Kannel 1989) and is over 60% in those with the most severe disease (CONSENSUS Trial Group 1987); the one year mortality in patients with NYHA IV symptoms is approximately 50% compared to a similar rate at 3-5 years in mild heart failure (McKee et al 1971, Cohn et al 1986). More recent data suggests that the age-adjusted, in-hospital mortality has fallen in recent years (Reitsma et al 1996).

2.4.2 Mode of death in CHF

It is not simply the presence of left ventricular dysfunction which confers the increased mortality risk in CHF. From the SOLVD prevention trial it is estimated that, among patients with impaired left ventricular dysfunction, the development of overt heart failure increases the risk of death 5-fold compared to patients with similar left ventricular function but no clinical evidence of heart failure (The SOLVD investigators 1992).

However it is important to note that asymptomatic left ventricular dysfunction carries an annual mortality of 3-4% and will progress to symptomatic disease in the majority of patients. Recent studies, in particular the prevention arm of SOLVD, indicate that substantial benefits in terms of development of symptomatic disease, reduced mortality and morbidity can be obtained by ACE inhibitor treatment in asymptomatic CHF (The SOLVD Investigators 1992). Mortality in CHF patients results from both cardiovascular and non-cardiovascular events (The SOLVD investigators 1991). The proportion of deaths attributable to cardiovascular causes increases as left ventricular ejection fraction falls (The SOLVD investigators 1991). The rate of mortality in CHF appears to be fairly consistent irrespective of the aetiology of the condition (Burgraff and Parker 1975, Cohn 1982, Demakis and Proskey 1974) although information from large prospective studies is sparse in this regard.

The presence of CHF is a powerful predictor of sudden cardiac death which becomes increasingly common with greater severity of disease: half of patients with ejection fraction < 30% will die suddenly, presumably from cardiac arrhythmia, within three years (Packer 1985). Half of the deaths in patients with heart failure in the Framingham study were "sudden" (Kannel et al 1988). The reason or reasons for the increased risk of sudden death in CHF is not clear but a number of mechanisms related to myocardial structure, drug therapy, electrolyte disturbance and neurohormonal activation have been proposed (Packer 1987). Although the arrhythmic nature of these deaths is difficult to establish in most cases, there is no doubt that the incidence of ventricular dysrhythmia is increased in CHF. The electrophysiological changes associated with myocardial remodelling predispose to dysrhythmia (Hansen et al 1990). In patients with CHF, the incidence of rhythm disorders is increased in patients with ventricular dilatation

(Sogaard et al 1994). Further to this, data from a substudy of the SOLVD trial indicate that once left ventricular ejection fraction is reduced below 35%, the occurrence of ventricular dysrhythmia correlates directly with ventricular size (Koilpillai et al 1996).

2.4.3 Indicators of prognosis

Upwards of 40 variables have been identified as prognostic indicators in CHF. The occurrence of cardiac arrhythmias confers a worse prognosis (Middlekauf et al 1991). Neurohormonal criteria pertaining to increased activity of the sympathetic nervous system (Cohn et al 1984, Rector et al 1987), and the renin angiotensin system (Rockman et al 1989) and factors relating to electrolyte imbalance such as hypo- natraemia (Lee and Packer 1986) are of relevance.

Of clinical criteria, the New York Heart Association (NYHA) functional class is directly related to sudden death in CHF, death being much more likely for those in functional class IV (McKee et al 1971, Swedberg 1987, Wilson et al 1983). As the NYHA classification system depends upon exercise capacity for stratification, it is not surprising that this too is also a good predictor of prognosis in CHF, as is left ventricular ejection fraction (Cohn et al 1987, Gradman et al 1989). However the relationship between systolic dysfunction and exercise capacity is unclear for any individual patient (Higginbotham et al 1983, Meiler et al 1987). Plasma noradrenaline levels (Cohn et al 1984, Swedberg et al 1990) and plasma renin activity are powerful predictors of mortality in severe CHF (Lee & Packer 1986). As noted above, the activity of both the sympathetic nervous system and renin-angiotensin system is increased in CHF. Catecholamines may be directly arrhythmogenic or may contribute to electrolyte disturbances in CHF (Struthers et al 1983). Angiotensin II may enhance sympathetic activity or may itself contribute to

electrolyte disturbances (Horton & Biglieri 1962).

With regard to the ACE inhibitors, captopril and enalapril reduce the frequency and severity of ventricular arrhythmias compared to placebo (Cleland et al 1984, 1985, Webster et al 1985). A number of the large trials in heart failure have demonstrated a reduction in sudden cardiac death related to ACE inhibitor therapy (Cohn et al 1991). The mechanism of the improvement in mortality obtained from the ACE inhibitors in CHF remains unclear but may be attributable to any one, or more likely, a combination of the known effects of these drugs in CHF: direct antiarrhythmic effects, decreased ventricular volume, reduced ventricular dilatation (Konstam et al 1991), retention of potassium (Packer & Lee 1986), parasympathomimetic effects and reduction in myocardial interstitial fibrosis (Brilla et al 1991). To what extent the improvement in loading conditions or alternatively neuroendocrine blockade contribute to these effects of ACE inhibition is at present unclear.

There is conflicting evidence on the relationship of aetiology of CHF to prognosis. The Framingham Study suggested that CHF of ischaemic aetiology holds a better prognosis than "idiopathic" cardiomyopathy (Ho et al 1993a). However the VHeFT-I study (Cohn et al 1986) and others (Franciosa et al 1983) suggest higher mortality in ischaemic cardiomyopathy. Such differences may be explained by the use of different diagnostic criteria for the presence of coronary artery disease.

2.5 DETERMINANTS OF EXERCISE CAPACITY IN CHF

2.5.1 Systolic function

Given that exercise capacity and left ventricular ejection fraction are important prognotic indicators in CHF, it is perhaps surprising that indices of left ventricular function

correlate poorly with exercise capacity (Baker 1984, Franciosa et al 1981, Meiler et al

1987). Approximately 50% of the variance in exercise capacity in CHF can be accounted

for by changes in maximal heart rate and cardiac output at peak exercise (Myers &

Froelicher 1991), leaving 50% unaccounted for. In CHF, a downward shift occurs in the

Frank-Starling curve relating stroke volume to left ventricular preload. Any increase in

preload such as occurs in exercise is not met with a sufficient rise in stroke volume, a

defect which leads eventually to left ventricular dilatation, increased afterload, increasing

pulmonary venous pressure, and right ventricular dilatation and failure. Treatment of

systolic dysfunction is therefore based on reducing preload with diuretics and venodilator

agents, reducing afterload with arterial vasodilators and to a lesser extent improving

myocardial contractility, for example with inotropic agents.

2.5.2 Diastolic function

A significant proportion of patients with symptoms of heart failure have normal

resting left ventricular systolic function, accounting for up to one third of referrals to

specialist centres in the U.S.A. (Dougherty et al 1984, Marantz et al 1988). This implies

that diastolic filling abnormalities may be relevant to the disease state in many patients and

agrees with the finding of a close association between indices of ventricular filling and

exercise tolerance in heart failure (Higginbotham et al 1983, Szlachcic et al 1985). In

patients with ischaemic heart disease and mild to moderate systolic dysfunction there is a

strong association between exercise capacity and diastolic filling abnormalities during

exercise (Lele et al 1996). The abnormal LV diastolic filling of CHF is characterised by

heterogeneity of mitral valve inflow patterns (Lavine & Arends 1989). In patients with

CHF, Doppler flow pattern across the mitral valve is a better predictor of symptomatic

status than left ventricular ejection fraction (Xie et al 1996). Both the above studies confirmed a poor association between either resting or exercise indices of systolic function and exercise tolerance.

In patients with ischaemic heart disease (Nonogi et al 1989) and dilated cardiomyopathy (Sato et al 1993) there is an abnormal upward shift in the diastolic component of the left ventricular pressure/volume relationship during exercise. Although the ventricle with isolated diastolic dysfunction can increase stroke volume, reduced ventricular compliance leads to limitation of cardiac output during periods of rapid ventricular filling as occurs during exercise. The level of diastolic dysfunction may be fixed, in the case of myocardial fibrosis or myocyte hypertrophy (Grossman 1990) or may be exacerbated by myocardial ischaemia (Carroll et al 1988). Myocardial ischaemia results in a marked reduction in the rate of isovolumic relaxation as a result of reduced intra-cellular levels of cyclic AMP. In patients with exercise induced ischaemia, abnormalities of diastolic function occur earlier than those of systolic function (Feldman et al 1987). Changes in filling pressure on the right side of the heart relate to improvements in exercise tolerance (Wilson & Ferraro 1982) and high ventricular filling pressure has been related to the reduction in exercise capacity (Pouleur et al 1990). The improvement in exercise capacity seen with the beta-adrenoreceptor agonist xamoterol in the study of Pouleur was associated with an improvement in the diastolic pressure-volume curve.

All of the disease states commonly associated with CHF will, if inadequately treated, lead to reductions in ventricular compliance associated with diastolic dysfunction. As recently reviewed, the identification and interpretation of parameters of diastolic dysfunction is difficult and complex (Brecker & Gibson 1996). With particular regard to the ACE inhibitors, interest in the future will centre on whether these drugs can improve

Table 2.3

Factors affecting exercise capacity in CHF.

Central mechanisms
        Systolic function
        Diastolic function
        Pulmonary haemodynamics

Peripheral factors
        Regional blood flow abnormalities
        Skeletal muscle abnormalities
        Vasodilatory limitation

Pulmonary factors
        Ventilation-perfusion mismatch
        Physiological dead space
        Pulmonary artery pressure
        Respiratory control

exercise capacity and prognosis in patients with isolated diastolic dysfunction, given their proven efficacy in all degrees of symptomatic and asymptomatic systolic dysfunction.

2.5.3 Pulmonary function and haemodynamics

Abnormalities of respiratory function are found in a significant proportion of CHF patients. In a large series of patients being evaluated for heart transplantation, abnormal respiratory function tests were found in 67% (Wright et al 1990). Abnormalities of diffusion capacity, forced expiratory volume (FEV_1), the ratio of FEV_1 to vital capacity (VC), minute ventilation and airway flow have been described. Respiratory function is often normal at rest with changes taking place during exercise. Indeed cardiopulmonary exercise testing allows accurate assessment of maximal exercise capacity in CHF (Weber & Janicki 1985). Maximal oxygen uptake during exercise (VO_2 max) is a strong predictor of prognosis (Cohn et al 1987).

A number of variables, such as resting pulmonary capillary wedge pressure, resting mean pulmonary artery pressure and resting total pulmonary resistance have been reported to correlate better with VO_2 max than any systemic haemodynamic parameter (Franciosa et al 1985). Other studies have failed to find any correlation between peak pulmonary wedge pressure during exercise and exercise tolerance (Fink et al 1986). Pulmonary hypertension is an adverse prognostic factor in CHF and exercise capacity is increased by improvements in pulmonary haemodynamics (Pellicia et al 1993). However there is no correlation between pulmonary haemodynamic measurements and the ventilatory response to exercise (Fink et al 1986). It has been proposed that a minimum level of cardiac output in response to exercise is necessary to perfuse all areas of the lungs to prevent ventilation/perfusion mismatch (Davies et al 1992).

Compared to normal individuals, increased ventilation relative to workload (Sullivan et al 1988) is seen in patients with CHF and a high ratio of minute ventilation to CO_2 production (VE/VCO_2) reflects the higher than normal ventilatory cost of CO_2 excretion during exercise (Buller & Poole-Wilson 1990, Lewis et al 1992). This increase in turn reflects a degree of ventilation/perfusion mismatch (Buller & Poole-Wilson 1990, Davies et al 1991). The VE/VCO_2 slope is inversely related to exercise tolerance and to VO_2 max (Buller & Poole-Wilson 1990) but appears not to correlate with haemodynamic variables (Fink et al 1986, Sullivan et al 1988). A subgroup of patients with CHF appear to have a high VE/VCO_2 ratio at rest, implying improvement of the expected ventilation-perfusion mismatch (Wada et al 1993). However impairment of the ability to maintain ventilation-perfusion matching on exercise appears to exist (Uren et al 1993a), perhaps due to the enhanced state of matching at rest. Although VO_2 max predicts prognosis in CHF, the clinical applicability of this measure is limited by the inability of a great many CHF patients to achieve VO_2 max. Recently changes in the VE/VCO_2 ratio with early exercise have been shown to correlate with VO_2 max and thus serve as a predictor of the severity of CHF (Milani et al 1996). Moreover, these investigators demonstrated that a reduction in the VE/VCO_2 ratio of < 10% during early exercise predicts severely impaired functional capacity.

The VE/VCO_2 slope in patients with CHF has been compared to that in patients with coronary artery disease and normal resting left ventricular function, pacemaker dependent patients, and normal controls (Banning et al 1995). Patients with normal left ventricular function at rest all had exercise induced myocardial ischaemia and dysfunction comparable to that of the CHF patients. While the expected high VE/VCO_2 slope during exercise was apparent in the CHF patients, the slope in the patients with ischaemic heart

disease was normal. This suggests that the ventilation/perfusion mismatch in CHF is not due to acute limitation of pulmonary blood flow during exercise. Moreover, in pacemaker dependent patients with left ventricular dysfunction, the abnormal VE/VCO_2 slope was increased further if the exercise induced increase in cardiac output was limited by fixed rate as opposed to rate responsive pacing. Such dependence on heart rate was not seen in pacemaker dependent patients with normal LV function. This latter finding is not consistent between studies, the VE/VCO_2 ratio being reported as abnormal in pacemaker dependent patients with apparently normal LV function (Tani et al 1992).

There is no doubt that acute bronchospasm occurs in patients with acute pulmonary oedema and resolves with resolution of the oedema. Inhaled broncho dilators appear to improve FEV_1 and VO_2 max (Uren et al 1993b) although some investigators have been unable to show such a response (Moore et al 1993). However in treated CHF abnormalities of airway function persist. The reduction in FEV_1 and VC parallels the severity of the patients' symptoms (Puri et al 1994) and FEV_1 correlates well with VO_2 max. The bronchoconstrictor response to inhaled methacholine in patients with CHF is abolished by inhalation of the vasoconstrictor methoxamine (Cabanes et al 1989). It has thus been suggested that high left ventricular filling pressure causes transudation of plasma from bronchial wall vessels thereby causing bronchial hyper-responsiveness.

In summary,abnormalities of both pulmonary haemodynamics and airway function exist in CHF. These appear to represent the consequences of alterations in pulmonary ventilation-perfusion relationship at rest, chronic changes in bronchial blood vessel structure, altered airway function, and impaired ability of the cardiorespiratory unit to respond appropriately to the stimulus of exercise.

2.5.4 Regional blood flow

Cardiac output is reduced in CHF both at rest and during exercise (Sullivan et al 1989, Weber & Janicki 1985). Marked abnormalities occur in regional blood flow, the degree of disturbance being directly related to the severity of CHF (Zelis & Flaim 1982). Flow to the coronary and cerebral vasculature is preserved longest but during exercise there is an early reduction of flow to the skeletal muscle circulation (Zelis & Flaim 1982). Blood flow to exercising tissue is disproportionately reduced in CHF (Sullivan et al 1989).

2.5.5 Skeletal muscle abnormalities

Although a number of skeletal muscle abnormalities occur CHF, such changes are also seen in muscles deconditioned for reasons other than heart failure (Kayanakis 1989). The finding that VO_2 max and anaerobic threshold improve after cardiac transplantation has been taken by some authors to indicate that the skeletal muscle abnormalities in CHF are secondary to altered central haemodynamics (Davies 1993) and by others to indicate "localised deconditioning" unrelated to reduced cardiac output (Myers et al 1991). While some studies have demonstrated impaired vasodilatory reserve in CHF (Le Jemtel et al 1986, Zelis et al 1968), others have suggested that the failure of skeletal muscle blood flow to increase during exercise is due to a reduction in the driving pressure rather than to limitation of vasodilatation (Wilson et al 1986, 1988).

Closely allied to the changes in regional blood flow seen in CHF are alterations in skeletal muscle biochemistry. Phosphorous-31 magnetic resonance imaging (31P-MRI) has been used to study the biochemistry of working muscle. In patients with CHF the rapid development of acidosis and depletion of skeletal muscle phosphocreatine levels have been

observed in-vivo in comparison to controls (Massie et al 1987, Rajagopalan et al 1988).

This and other metabolic abnormalities in CHF, such as greater glycolysis, appear not to

be wholly explained on the basis of reduced muscle blood flow (Massie et al 1987).

Histological studies of skeletal muscle from patients with CHF have revealed a variety of

changes, none of which are consistent across studies. Many suggest a shift from aerobic to

anaerobic metabolism. The changes include atrophy of type II fibres, an increased

percentage of type II B fibres and an increased percentage of type I fibres (Drexler et al

1992). Increased lipid content (Lipkin et al 1988) and decreased levels of mitochondrial

enzymes (Sullivan et al 1990) are also found.

Lindsay and colleagues recently demonstrated the presence of a number of

histological abnormalities in skeletal muscle and diaphragm biopsies from patients with

heart failure of either ischaemic or idiopathic aetiology (Lindsay et al 1996). These authors

used a number of monoclonal antibodies to neonatal myosin and to fast and slow myosin

heavy chains to establish fibre type and to establish the presence of abnormal myosin. A

number of the abnormal findings suggested fibre metaplasia and regeneration: neonatal

myosin was present 7 of the 17 patients; a number of biopsies showed internal nuclei,

tubular aggregates or subsarcolemmal deposits. Of particular note was the presence of

central cores in type I fibres from 4 of 10 dilated cardiomyopathy patients and 1 of 7 with

ischaemic cardiomyopathy. Although no consistent changes were found, it is of interest

that abnormalities were more marked in subjects with dilated compared to those with

ischaemic cardiomyopathy. In addition, myopathic changes were found in all muscles

biopsied (vastus lateralis, pectoralis major and sternothyroid) but were most severe in the

diaphragm. No such changes were seen in biopsies from patients with normal left

ventricular function but restricted exercise capacity due to ischaemic heart disease,

suggesting that the myopathic features in the CHF patients were specific to CHF. The only other study to have compared the histology of skeletal muscle biopsies from patients with CHF of differing aetiologies failed to show any differences, although immuno-histological methods were not used (Mancini et al 1989).

The skeletal muscle of patients with CHF shows reduced activity of the oxidative enzymes citrate synthetase and 3-hydroxyacyl-CoA dehydrogenase compared to the muscle of normal individuals (Schaufelberger et al 1996). In the same study, muscle biopsies taken at maximal exercise showed increased levels of adenosine triphosphate, creatine phosphate and glycogen with reduced glucose and lactate concentrations. It is also of note that there was no correlation between haemodynamic variables and levels of oxidative enzymes. On the basis of their findings, the authors concluded that neither skeletal muscle substrate content nor lactate accumulation are limiting factors for exercise performance in CHF. The authors also concluded that the oxidative capacity of skeletal muscle is reduced in CHF, leading to a reduction in effective muscle mass. Similar results were obtained in a recent study using ^{31}P magnetic resonance imaging during exercise in patients with CHF (Kemp et al 1996). Maximum oxidative capacity was improved by exercise training in this study.

Thus a plethora of abnormalities exist which may contribute to reduced exercise capacity in CHF. Reduced metabolic efficiency results from macroscopic and microscopic muscle fibre changes as well as altered mitochondrial numbers and function. Marked histological changes seen in the diaphragm (Lindsay et al 1996) may help to explain the correlation between the sensation of dyspnoea and the amount of diaphragmatic work performed (Mancini et al 1992). A recent study showing that the determinants of exercise capacity in CHF change, with the development of cardiac cachexia, from the age of the

patient and muscle mass to peak skeletal muscle blood flow (Anker et al 1997). This finding may explain at least some of the disparate findings of previous studies.

2.5.6 Neurohormonal response to exercise in CHF

Relative vasoconstriction is known to exist in CHF, at least partly in response to the increased activity of the sympathetic nervous and renin-angiotensin systems. Elevated levels of noradrenaline, renin and a number of other neurohormones are associated with poor prognosis in CHF (Cohn et al 1984). Levels of plasma catecholamines are elevated in heart failure, particularly after initiation of diuretic therapy. Increases in total body (62%) and in cardiac (277%) noradrenaline spillover rates have been demonstrated in patients with severe heart failure (Kaye et al 1994). The role of elevated catecholamine levels in the pathophysiology of heart failure is however unclear. Little in the way of haemodynamic change is seen after inhibition of noradrenaline synthesis (Franciosa & Schwartz 1989). Sympathetic neural activity to skeletal muscle is markedly increased in CHF (Ferguson et al 1990) and could theoretically interfere with metabolically driven vasodilatation during exercise. A significant positive correlation exists between skeletal muscle sympathetic nervous activity and left ventricular filling pressure (Leimbach et al 1986), suggesting a possible role for cardiopulmonary baroreceptors in the regulation of overall sympathetic activity.

Down-regulation of beta-adrenergic receptors occurs in the human myocardium in CHF (Colucci et al 1989) and abnormal baroreceptor reflexes are apparent in animals with CHF (Higgens et al 1972). In contrast to the elevated levels of catecholamines at rest, the sympathetic response to exercise may be abnormal in CHF. For a given uptake of oxygen the blood pressure response in CHF is normal. However when expressed as a percentage

of maximal oxygen uptake, the response is less than seen in normal subjects (Francis 1987).

Activation of the renin-angiotensin system occurs in heart failure, again particularly after initiation of treatment with diuretics (Massie et al 1988). The importance of activation of this system in CHF is suggested by the efficacy of the ACE inhibitors in improving exercise tolerance. However the observation that vasodilators other than the ACE inhibitors improve exercise tolerance in CHF indicate that mechanisms other than inhibition of an activated renin-angiotensin system play a role. The proposed interaction between the sympathetic nervous system and the renin-angiotensin system may be of relevance and increased activity of both systems is likely to contribute to the decreased vasodilatory reserve in heart failure. Angiotensin II stimulates the release of noradrenaline from cardiac sympathetic nerves in animals (Blumberg et al 1975). However blockade of the renin-angiotensin system does not improve leg blood flow acutely (Wilson & Ferraro 1985).

Data from the CONSENSUS study indicates that the degree of neuroendocrine activation correlates with the beneficial response to ACE inhibition in CHF. It would appear that exercise tolerance is limited in CHF at least partly as a result of a combination of abnormalities leading to skeletal muscle hypoperfusion. Exercise capacity and skeletal muscle oxygen utilisation do not improve immediately after an improvement in haemodynamics, suggesting that intrinsic abnormalities of skeletal muscle in CHF contribute to the limitation of exercise capacity in this condition. The observation that physical training improves skeletal muscle metabolism has been taken to indicate that muscle deconditioning is important in the reduction in exercise tolerance seen in CHF (Adamopoulos et al 1993).

Activation of the sympathetic nervous system and renin-angiotensin system in CHF has the purpose of maintaining arterial blood pressure. As noted above, such activation is exacerbated by diuretic therapy. Studies of the neurohormonal response to exercise in CHF in the absence of diuretic therapy are rare. In a recent study, Ferrari et al studied the neurohormonal response to exercise and standing in response to mild exercise in patients with severe untreated CHF due to myocardial disease or chronic constrictive pericarditis (Ferrari et al 1996). These authors found that compared to normal control subjects, patients with CHF showed greater heart rate and plasma levels of noradrenaline, renin activity, aldosterone, cortisol and ANP. Levels were elevated in the patient groups both at rest and during gentle exercise. Similar neurohumoral responses were seen to exercise and to standing in both patient and control subjects. In response to exercise, patients with CHF due to constrictive pericarditis showed a greater rise in heart rate and those with CHF due to myocardial disease showed a greater rise in plasma renin activity. Thus the response to standing and to gentle exercise appeared to be largely normal although superimposed on abnormal resting conditions.

2.5.7 The effect of ACE inhibition on exercise tolerance

As reduction in exercise capacity is such a prominent symptom and exercise capacity an important prognostic indicator in CHF, exercise testing is often used to assess work capacity and the effect of intervention (medical or surgical) on this parameter. Changes in exercise capacity are often used as part of the assessment of the effect of therapy on quality of life. A number of different protocols have been used and doubts have been cast on the relevance of at least some of these to the impact of therapy on patients' incapacity during routine daily activities (Walsh et al 1995).

There is no doubt that the ACE inhibitors improve mortality in CHF. However the benefit of these agents in terms of improved lifespan is modest. In many patients the main benefit of the use of these agents is in improved morbidity in terms of exercise capacity and relief of symptoms. This is particularly so for those patients with more severe CHF in whom the improvement in mortality from ACE inhibition can be measured on average in terms of a few months. It is for this reason that many clinical studies of pharmacological intervention in CHF have formally assessed exercise tolerance using specific exercise protocols. A number of studies have suggested that changes in exercise capacity following ACE inhibition correlate poorly with mortality and morbidity benefits, and also with patients' perceptions of symptoms. In the V-HeFT II study exercise capacity was improved to a greater extent by the combination of isosorbide dinitrate and hydralazine than by ACE inhibition (Cohn et al 1991). Similarly no effect on exercise capacity was seen with ramipril although symptoms did improve (Gundersen et al 1994). However studies of the changes in exercise capacity following ACE inhibition in CHF have used a variety of protocols, were of a number of different designs, patient characteristics, study size and duration of follow up. Thus differences in the findings with regard to exercise capacity are perhaps unsurprising. A recent review of this area concluded that ACE inhibitors improve exercise capacity and symptoms in patients with CHF (Narang et al 1996). In the vast majority of trials of ACE inhibition in CHF there was good agreement between the effect of therapy on both symptoms and exercise capacity. A number of factors including study size, duration of follow up and method of exercise testing were found to affect outcome. Interestingly, complex measurements of gas exchange and oxygen consumption appeared to add little to the simple assessment of exercise capacity using defined exercise protocols. From this review it appears that adequately powered

studies with prolonged (> 4-6 months) follow up are required for the proper evaluation of changes in exercise capacity following intervention in CHF.

Recently the importance of the peripheral circulation and tissues to abnormalities of exercise capacity in CHF has become apparent. A number of central and peripheral abnormalities have been shown to affect exercise capacity in CHF (Table 2.3). However the relative importance of these factors remains largely unresolved. Moreover the relative importance of cardiac, pulmonary and peripheral circulation factors defining the patients perception of breathlessness are complex (Wasserman & Casaburi 1988).

2.6 THE TREATMENT OF HEART FAILURE

2.6.1 General

The results of clinical trials conducted over recent years have indicated that the progression of heart failure can be slowed by physical exercise (Coats et al 1990) resulting in measurable improvements in physiological abnormalities such as skeletal muscle metabolism (Adamopoulos et al 1993). All patients with heart failure require control of fluid balance. In the early stages of the disease this can be achieved by restriction of dietary fluid and sodium intake. Although drug treatment initially consists of the use of diuretics, there is now an established case for the addition of an ACE inhibitor at as early a stage as possible after diagnosis of CHF.

2.6.2 Pharmacological treatment of CHF

2.6.2.1 Diuretics

The use of diuretics reduces ventricular preload thereby reducing ventricular size

and wall stress. All patients with systolic ventricular dysfunction and many with diastolic dysfunction will require a diuretic for management. This generally requires the use of loop diuretics; in more severe heart failure the combination of a loop diuretic with a thiazide and /or an aldosterone antagonist may be required. The efficacy of diuretics in heart failure depends upon adequate natriuresis, and the combination of thiazide with loop diuretic is especially potent in this regard.

2.6.2.2 Positive inotropic agents

All inotropic agents so far identified exert their effect by increasing the availability of intracellular free calcium. Two of the major classes of inotropic agent, the phosphodiesterase inhibitors (PDI) and the β-adrenoceptor agonists, act to increase availability of the nucleotide cyclic AMP (cAMP), higher levels of which increase calcium influx at a given level of cell membrane depolarisation. Inotropic agents can be classified according to their dependence or otherwise on cAMP.

<u>cAMP-independent inotropic agents</u>

Digitalis glycosides

The treatment of heart failure has long had as one of its cornerstones the use of the digitalis glycosides, in particular digoxin. The extent of the use of these compounds for the treatment of heart failure now varies widely depending on the standard practice of the individual physician or the accepted treatment of heart failure on a regional or national basis. Marked differences in patterns of its use in CHF are also seen within the same country. For example in the SOLVD treatment trial only 60% of CHF patients with ejection fraction less than 35% were receiving digoxin at entry to the study (The SOLVD

investigators 1991). Digitalis glycosides are however relatively mild inotropic agents.

Limited data exists in terms of the effects of digoxin on symptoms and exercise capacity in

CHF. Published results on the effect of digoxin on exercise capacity in CHF are

conflicting, with beneficial (DiBianco et al 1989) and neutral results (Fleg et al 1991)

being reported. An increase in ventilatory oxygen intake following digoxin therapy has

been reported (Sullivan et al 1989). Morisco et al have utilised ambulatory radionuclide

monitoring to study the effect of digitalis as compared to placebo on left ventricular

function in CHF (Morisco et al 1996). Treatment with digitalis for 3 weeks elicited

normalisation of the heart rate response to exercise and improvements in both left

ventricular end-systolic and end-diastolic volumes during exercise and in ejection fraction

at rest and during exercise. Interestingly, the improvements in hamodynamic parameters

were largely independent of changes in heart rate.

There is evidence of both short-term and sustained haemodynamic benefits of

digoxin in CHF (Arnold et al 1980). While the drug is undoubtedly of benefit in atrial

fibrillation, its efficacy in sinus rhythm has been questioned. A number of uncontrolled

trials have shown that withdrawal of digoxin from patients with stable CHF does not lead

to adverse clinical effects (Dall 1970, Fonrose et al 1974, Hull & Makintosh 1977).

However a number of controlled trials have reached the opposite conclusion. A meta-

analysis of 7 such trials involving 617 patients with CHF in sinus rhythm found a

significant benefit from continuation of digoxin compared to its withdrawal (Jaeschke et al

1990). These studies were limited by a number of factors, in particular by the fact that they

studied the effects of the withdrawal of of digoxin rather than its commencement. In

addition the applicability of the results to current practice in the treatment of CHF is

unclear in that the patients were on the whole not receiving an ACE inhibitor. Two recent

studies have addressed the issue of the effect of withdrawal of digoxin from the drug

regimen of patients with stable CHF (Packer et al 1993, Uretsky et al 1993). The results of

both trials were similar, with decreased exercise capacity and greater frequency of

treatment failure in those patients randomised to withdrawal of digoxin. Both studies were

however small, with 100 and 178 patients randomised. None of these trials was designed

to study the effects of digoxin on mortality in CHF, about which concerns have been

expressed (Yusuf et al 1992). Information on the effects of digoxin on mortality is awaited

from the results of two ongoing clinical trials, the Digoxin Investigation Group, and V-

HeFT III (Yusuf et al 1992).

In terms of the symptomatic improvement elicited by digoxin in CHF, positive

inotropism may be of secondary importance to other properties of the drug. Digoxin has a

number of effects on autonomic function, causing vagal enhancement and impairment of

sympathetic outflow, and there is enhanced sensitivity of barorereflexes. Acute inhibition

of the activity of the renin-angiotensin-aldosterone axis is also seen. The net result is

vasodilatation, which is of course in itself likely to be of benefit in CHF.

Calcium-sensitising agents: vesnarinone and pimobendan

A number of agents have been identified which in addition to inhibiting

phosphodiesterase have a variety of additional effects which may potentially explain

their inotropic properties. Of these agents two have reached the stage of clinical trials

in heart failure. Pimobendan (UDCG-115) increases the sensitivity of the contractile

apparatus to calcium in vitro (Fujino et al 1988) and in heart failure decreases in

systemic vascular resistance and MAP are seen with concomitant increases in stroke

volume, cardiac index and heart rate (Hasenfuss et al 1989). The compound also has

cAMP phosphodiesterase inhibiting activity. Vesnarinone has multiple effects on ion channels (Lathrop et al 1989, Yatani et al 1989), any of which may increase intracellular calcium concentration.

Although the overall experience with PDIs in CHF has been unfavourable (vide infra), a number of studies with each of these two compounds have produced encouraging results for their use in CHF. A number of small trials demonstrated a beneficial effect of pimobendan on exercise capacity (Katz et al 1992, Kubo et al 1992), haemodynamics (Hasenfuss et al 1989) and quality of life (Kubo et al 1992) in CHF. Although the larger Pimobendan in Congestive Heart Failure (PICO) trial confirmed the effects of this compound on exercise capacity, no benefits were evident in terms of quality of life and there was a trend towards increased mortality in the pimobendan group (The Pimobendan in Congestive Heart Failure (PICO) Investigators 1996). It has been suggested, on the basis of meta-analysis of a number of small trials, that vesnarinone may have a beneficial effect in CHF (Nony et al 1994). Of the 3 studies with vesnarinone, two involved a total of 138 patients with 1 vesnarinone and 8 placebo deaths and were insufficiently powered to detect a meaningful mortality difference (OPC-8212 Research Group 1990, Feldman et al 1991). In a third placebo-controlled study of vesnarinone 60mg or 120 mg daily, the higher dose was associated with increased mortality compared to placebo while the lower dose was associated with a 62% reduction in all-cause mortality (Feldman et al 1993). There was in addition a reduction in the rate of progression of heart failure and in quality of life. However the VEST trial, comparing the effect of addition of vesnarinone 30 mg or 60 mg in CHF, was stopped early in 1996 due to increased mortality in both active treatment groups compared to placebo.

cAMP-dependent inotropic agents

Phosphodiesterase inhibitors

Selective inhibitors of phosphodiesterase III (phosphodiesterase inhibitors, PDI's) combine positive inotropic with peripheral vasodilator properties. Many of these compounds produce short- and long-term haemodynamic improvement at rest and during exercise: this is the case for amrinone, milrinone, piroximone, and enoximone.

Unfortunately this effect has not translated into improved mortality; on the contrary, a number of the agents have been associated with increased mortality on long term treatment. Initial experience with PDI's in the treatment of CHF was in the setting of uncontrolled clinical trials. These on the whole demonstrated haemodynamic improvement but the incidence of side-effects was often high. Reported 1-year mortality varied widely but was on average around 75%, comparing unfavourably with trials of standard vasodilator therapy in CHF (Packer & Leier 1987). In controlled trials milrinone was associated with a 34% increase in the risk of death from cardiovascular causes (Packer et al 1991). Similar results were obtained with enoximone (Uretsky et al 1990).

Flosequinan

Flosequinan, like vesnarinone, is a quinolone derivative and a weak PDI. This drug was marketed for the treatment of CHF but withdrawn by the manufacturer following an increase in mortality associated with its use (Boots Company 1993). The inotropic effect produced by flosequinan in animal and in vitro studies is dose related and not apparent in the accepted therapeutic range (Yates 1991). The mechanism of the vasodilator effect of flosequinan is not fully understood. In isolated cardiomyocytes, flosequinan is chronotropic at therapeutic concentrations, positive inotropism and PDI

activity being seen only at higher concentrations (Kelso et al 1995).

In spite of the apparent deleterious effects on mortality seen with a number of positive inotropic agents, significant beneficial effects on quality of life have been observed in a number of trials of these agents in CHF, e.g., enoximone (Cowley & Skene 1994). Given that the poor prognosis for heart failure, particularly in more severe cases, there may be a place for agents which can improve quality of life in spite of adverse effects on mortality.

Catecholamines

Both β1 and β2 adrenoceptors are found in human myocardium, the β1 subtype predominating in ventricular tissue. Stimulation of either subtype leads to increased cAMP levels via activation of adenylate cyclase. Catecholamines have a direct chronotropic as well as inotropic effect. Beta receptor agonists also have a lusitropic effect, i.e., they increase the rate of cell relaxation. While activation of the sympathetic nervous system is a compensatory mechanism in early heart failure, progressive sympathetic activity appears to have deleterious effects in the long-term. The prognosis in CHF is directly related to the plasma noradrenaline level at presentation (Cohn et al 1984, Francis et al 1993). Chronic adrenergic stimulation leads to a reduction in the number (down-regulation) of cell surface β-receptors. The extent of reduction is directly related to the severity of heart failure (Fowler et al 1986). In the myocardium, preferential down-regulation of β1 receptors occurs.

Animal models of heart failure have demonstrated that intermittent sympathomimetic stimulation improves exercise capacity, reduces resting heart rate and improves heart rate during exercise. In addition, and perhaps significantly, plasma

noradrenaline concentration and renin activity are reduced. Unfortunately to date it has not been possible to translate encouraging animal experiments into beneficial effects in human heart failure. Initial studies of the effects of intermittent infusion of dobutamine in CHF resulted in improved haemodynamic parameters, symptoms and exercise tolerance. However a multi-centre trial comparing intermittent inotropic therapy via weekly infusion of dobutamine (48 hours per week) to placebo was halted prematurely due to a dramatic increase in mortality with active therapy (Dies et al 1984). Infusion of dobutamine in animal studies was over 1-2 hours while periods of 4-72 hours have been used in man. Down-regulation of β-receptors occurs only with continuous receptor stimulation for periods of greater than 4 hours (Tohmeh et al 1980). A regime of dobutamine infusion (20-25 mg/kg/min) of 30 minutes duration on 4 days per week for three weeks has been employed in patients with CHF (Adamopoulos et al 1995). This resulted in significant improvements in exercise capacity, reduction in peripheral vascular resistance and increases in lymphocyte β-receptor density, indicating receptor up-regulation. As in animal studies, plasma noradrenaline concentration fell. The observed changes mimic those seen in response to exercise.

Dopamine receptors (DA1) are present in the vasculature mediating arteriolar vasodilatation and inhibition of noradrenaline release from sympathetic nerves. The pharmacology of drugs acting at these receptors is complicated by alpha adrenergic agonism in some cases, e.g., ibopamine. Beneficial haemodynamic responses to these agents have been demonstrated (Dei Cas et al 1989) but haemodynamic tolerance has occurs after short-term intravenous administration of feldopam (Munger et al 1990) and down regulation of dopamine receptors with chronic usage has been postulated as a potential problem with these agents (Davies & Sheridan 1993). A recent placebo-

controlled study (PRIME II) of the effect of long-term oral therapy with the dopaminergic agonist ibopamine, on symptoms and mortality in patients with NYHA class III or IV heart failure was halted prematurely due to significantly increased mortality in patients receiving active therapy (D. Barnett, personal communication).

2.6.2.3 Vasodilator drugs

The central problem in CHF, whatever the aetiology, is failure of the heart to sustain adequate organ perfusion pressure. Neurohormonal activation in response to heart failure, while initially acting to re-establish homeostasis, in the long term causes further salt and water retention, exacerbating the problem. Vasodilator therapy is used in an attempt to reduce cardiac pressures by reducing preload using venous vasodilators, afterload using arterial vasodilators, or both using drugs with mixed properties or combinations of agents. At the time of writing, vasodilator therapy represents a vital part of the effective treatment of CHF.

Arterial vasodilators

By their effect on peripheral vascular resistance, arterial vasodilators act to reduce left ventricular afterload. Alpha adrenoreceptor antagonists such as prazosin, while reducing systemic blood presure and left ventricular filling pressures acutely, do not sustain these beneficial haemodynamic effects with chronic use in CHF (Bayliss et al 1985). Although a great many arterial vasodilators can be shown to have beneficial haemodynamic effects after acute administration to patients with CHF, the long-term efficacy of these drugs is related not simply to their acute haemodynamic effects. This has been demonstrated for the vasodilator combination of hydralazine plus isosorbide dinitrate

compared to enalapril in the V-HeFT study. Treatment with the ACE inhibitor resulted in a greater improvement in mortality in spite of the vasodilator combination giving a greater haemodynamic response and improvement in exercise tolerance (Cohn et al 1991).

Venous dilators

Venodilatation improves cardiac function in CHF by reducing preload. The standard group of venodilator drugs are the organic nitrates. As in the chronic use of these drugs in angina pectoris, tolerance to their haemodynamic effects occurs unless a nitrate free period is present (Elkayam et al 1991). Notwithstanding this problem, there is no doubt that the nitrates are of symptomatic benefit if given in adequate dosage in CHF. It has been suggested that haemodynamic tolerance to nitrate preparations may be prevented by the concomitant administration of diuretics (Mohanty et al 1995).

Mixed vasodilators

On pathophysiological grounds an effect on both cardiac preload and afterload in CHF is desirable. The only vasodilator therapy shown to improve prognosis in CHF is the combination of the arterial vasodilator hydralazine and the venodilator isosorbide dinitrate. In the first Veterans Administration Heart Failure Trial, V-HeFT I, this combination was associated with a 28% reduction in mortality compared to placebo over a mean follow up period of 2.3 years (Cohn et al 1986).

One of the most recently studied drugs with both arteriolar and venous dilator properties is the quinolone flosequinan. This drug has been shown to have a variety of effects on symptoms and exercise tolerance (Pitt 1991), positive inotropism dependent upon dosage (Perreault et al 1991), and reduced sympathetic and enhanced parasympathetic tone (Binkley et al 1992). As discussed above the drug has shown an

adverse effect on mortality in long term use and has recently been withdrawn, other than for use on a "named-patient" basis.

Calcium channel blockers

To date, studies of the use of calcium antagonists in CHF have been disappointing. The calcium antagonists are among the most effective vasodilator agents known. However well controlled studies with verapamil (Ferlinz & Gallo 1984), nifedipine (Elkayam et al 1990), diltiazem (Goldstein et al 1991) and minoxidil (Franciosa et al 1984) in CHF have shown treatment with these agents to be associated with an increased risk of death or worsening heart failure. The deleterious effects of the calcium antagonists may be due to their negative inotropic effects but activation of the sympathetic and renin-angiotensin systems may also play a part (Packer 1990). Newer drugs of this class may be less negatively inotropic (Kassis & Amtorp 1990) and in some studies have been shown to improve exercise capacity in patients with CHF (Dunselman et al 1989). The recently introduced agent lacidipine produces beneficial effects on cardiac output and peak oxygen consumption during exercise in comparison to placebo in patients with CHF (de Vries & Dunselman 1995). This was achieved without changes in heart rate or in neurohormonal parameters. A recent placebo-controlled study suggested that the calcium antagonist amlodipine may have beneficial effects on mortality and morbidity in severe CHF of non-ischaemic origin (Packer et al 1996). No benefit was seen in patients with ischaemic heart disease. Further developments are awaited but there is as yet little evidence to support the routine use of calcium antagonists as vasodilators in CHF.

2.6.2.4 Beta adrenoceptor antagonists

As discussed above elevated levels of plasma catecholamines are associated with a poor prognosis in CHF and down regulation of cardiac beta receptors is known to occur (Bristow et al 1982). Blockade of adrenergic activity to the failing myocardium has theoretical benefits in terms of reduced oxygen consumption and restoration of receptor density towards normal. The pooled results of all clinical trials of the effect of beta adrenoceptor antagonists after acute myocardial infarction provide powerful evidence of a beneficial effect (Yusuf et al 1985). The magnitude of the effect is of the order of a 20% reduction in mortality from long-term β-blockade after MI. There is also a similar reduction in nonfatal reinfarction among this group of patients. In-hospital, short-term β-blockade reduces early mortality by around 15% (ISIS-1, First International Study of Infarct Survival Collaborative Group, 1985). There appears to be little evidence of a greater effect of cardioselective β-blockers in this regard although it is of interest that those β-blockers with intrinsic sympathomimetic activity (ISA) appear relatively less efficacious in terms of outcome after acute MI (Yusuf et al 1985).

In spite of this evidence, the administration of a β-blocker in the setting of acute MI is by no means routine and the pattern of the use of these agents varies considerable both within and between countries. A multi-centre study studying variations in the treatment of acute MI in several thousand patients across 11 European countries revealed marked differences in the use of β-blockers in acute MI (Ketley & Woods 1995). The rate use of intravenous β-blockade ranged from < 1% in some countries to a maximum of 50%, and that of oral β-blocker use at discharge from 25% to >75%. Such wide variation is not seen in the use of other secondary preventive measures such as aspirin.

At least some of this variation can be explained on the basis of physicians' concerns

regarding the precipitation or exacerbation of heart failure in these patients (Braunwald et al 1983). The majority of trials of β-blockers following acute MI have excluded those with evidence of heart failure at entry. However two early studies which included such patients did not find any increase in mortality or in heart failure subsequent to inclusion (Balcon et al 1966, Clausen et al 1966). Indeed in terms of mortality the beneficial effects of β-blockade following MI have been shown to be greater in patients with CHF than in those without (Chadda et al 1986). The fear of acutely precipitating or exacerbating heart failure is often an overriding consideration to the physician attending the patient soon after acute MI, particularly where there is now unequivocal evidence of the long term benefits of ACE inhibition in this situation. This appears to have led to a relative decrease in the use of β-blockers after MI despite there being no evidence that these agents cause harm in the long term when there has been evidence of left ventricular dysfunction.

In addition to their efficacy in ischaemic heart disease, β-blockers have beneficial effects in dilated cardiomyopathy. Benefits are seen in terms of haemodynamic parameters (Anderson et al 1991), morbidity (Waagstein et al 1993) and mortality (Engemeier et al 1985). Although the largest of these trials (Waagstein et al 1993) failed to show any effect on mortality of the β-blocker metoprolol in dilated cardiomyopathy, all other primary endpoints were improved by active treatment. The improvements in left ventricular ejection fraction, echocardiogarphic fractional shortening and ventricular volume of 12 months of treatment with metoprolol are equivalent to those obtained with enalapril (Regitz-Zagrosek et al 1995).

Newer agents of the class have combined beta adrenoceptor antagonism with beta-2 agonist properties (celiprolol), or with alpha antagonism (carvedilol), the latter conferring peripheral vasodilatory properties in each case. Carvedilol in particular has

undergone extensive investigation and early experience with its use in CHF has been encouraging. The drug possesses antioxidant and antiproliferative effects in smooth muscle which may confer beneficial cardiovascular effects (Feuerstein & Ruffolo 1995). Carvedilol improves symptoms and both resting and exercise haemodynamics compared to placebo in patients with ischaemic or dilated cardiomyopathy (Das Gupta et al 1990, Krum et al 1995, Olsen et al 1995). A large, placebo-controlled trial of carvedilol as an adjunct to standard therapy in CHF has recently been terminated prematurely due to the finding of improved mortality with active therapy (Packer et al 1996). 1094 patients with CHF were randomised to receive placebo (n=398) or carvedilol (n= 696) in addition to standard therapy with digoxin, diuretic and ACE inhibitor. Over 6 months of follow up, mortality in the carvedilol group was 3.2% compared to 7.8% in the placebo group. A remarkable 65% reduction in risk was reported as well as 27% reduction in the risk of hospitalisation for cardiovascular causes, and a 38% reduction in the risk of the combined end-points of hospitalisation or death.

The place of β-blocker therapy in the routine management of CHF is by no means established. As discussed above there are theoretical grounds for their use in this setting and the evidence from early intervention trials is very encouraging. Further studies are required to confirm the above findings and to explore the potential benefit of these compounds in patients after acute MI. At the time of writing, large clinical trials which will study the effects of carvedilol in patients with left ventricular dysfunction after myocardial infarction, carvedilol in patients with severe CHF and carvedilol or metoprolol in all grades of CHF, are planned.

2.6.3 Angiotensin converting enzyme inhibitors

2.6.3.1.The role of ACE inhibitors in the treatment of heart failure

It has been known for many years that vasodilating drugs can be used to treat the signs and symptoms of CHF. However it was only relatively recently that the such effects were parallelled by improvement in mortality compared to placebo in patients with heart failure. The first such trial (the Veterans Administration Cooperative Vasodilator-Heart Failure Trial (V-HeFT I), Cohn et al 1986) demonstrated the improved mortality from the combination of hydralazine and isosorbide dinitrate. Of equal interest from V-HeFT I was the observation that another vasodilator, prazosin, had no beneficial effect on mortality. The finding from a later study, V-HeFT II, that the ACE inhibitor enalapril was superior to the combination of hydralazine and isosorbide dinitrate in terms of the effect on mortality (Cohn et al 1991) has led to extensive investigation of the effects and mechanisms of action of the ACE inhibitors in CHF and also to a dramatic change in the practice of the treatment of heart failure of all grades of severity.

A consistent finding of the trials involving the ACE inhibitors in heart failure has been that these drugs improve mortality (Table 2.4). As discussed above, the importance of the ACE inhibitors in the treatment of CHF can be gauged from the fact that trials of a number of alternative vasodilators in CHF, while demonstrating efficacy in terms of symptomatic improvement, have shown increased mortality compared to placebo. This is true for xamoterol (The Xamoterol in Severe Heart Failure Study Group 1990), milrinone (Packer et al 1991), amrinone (Massie et al 1985) and flosequinan (Boots Company 1993).

Interest in the use of ACE inhibitors in CHF was kindled following the demonstration of the beneficial effect on mortality of the vasodilator regimen of hydralazine/isosorbide dinitrate (Cohn et al 1986) and a number of placebo controlled
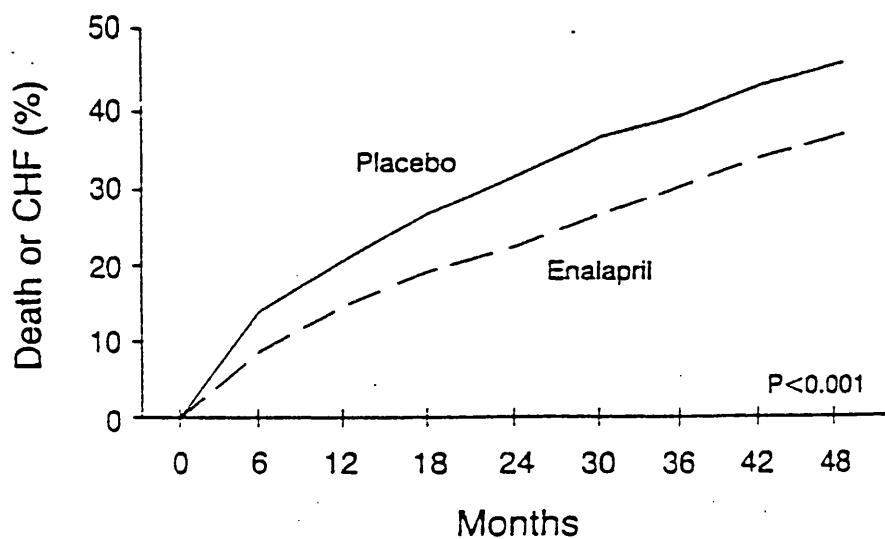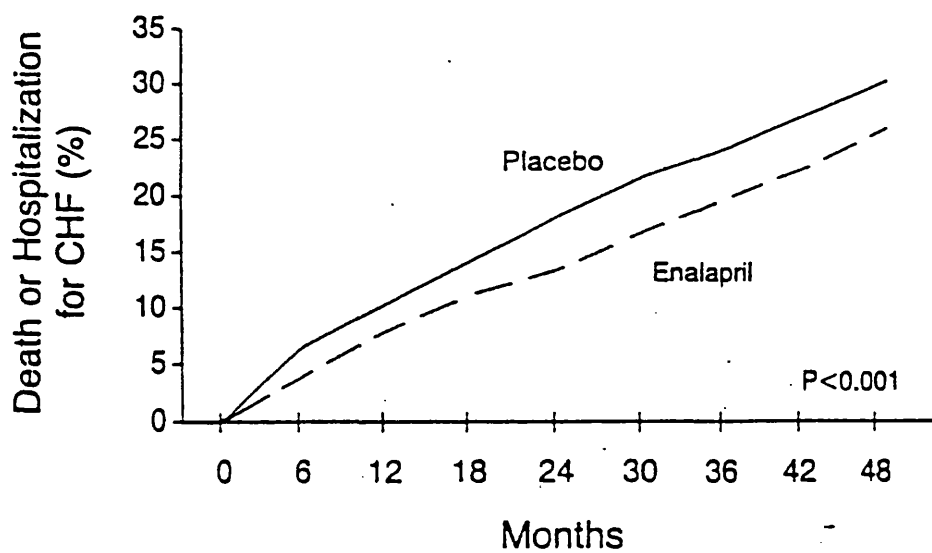
Figure 2.6.1:
Percentage of events in the SOLVD treatment (Top) and prevention (Bottom) trials. In the SOLVD events were defined as death or hospitalisation for CHF. In the prevention trial, events were defined as death or progress to CHF (from asymptomatic LV dysfunction).

trials which suggested a potential benefit from ACE inhibition (Furberg & Yusuf 1985).

The effect of an ACE inhibitor on mortality in CHF was first studied in the CONSENSUS

(Cooperative North Scandinavian Enalapril Survival Study) trial. Enalapril was shown to

improve mortality in patients with severe CHF, New York Heart Association functional

class IV (The Consensus Trial Study Group, 1987). A 31% improvement in 1-year

mortality was observed, with additional improvements in functional class and symptoms.

However at this time it remained common practice to restrict the use of ACE inhibitors to

patients with severe heart failure. Uncertainty about the use of the ACE inhibitors in mild

to moderate heart failure represented the origins of the SOLVD (Studies of Left

Ventricular Dysfunction) trial. This study randomised patients with left ventricular

ejection fraction of 35% or less to therapy with placebo or enalapril 2.5mg-10mg b.d. as

tolerated. Symptomatic patients requiring standard therapy for CHF, i.e., digoxin, diuretics

or vasodilator, were enrolled in the treatment trial, while asymptomatic patients were

enrolled in a prevention trial run in parallel. The end points in the treatment trial were

mortality, morbidity and hospital admissions due to heart failure, while in the prevention

trial the end point was the prevention of or delay in development of symptomatic or overt

CHF. Both arms of the trial demonstrated benefit from the ACE inhibitor (Figure 2.6.1).

The treatment trial (The SOLVD investigators 1991) showed an overall reduction in all-

cause mortality of 16% and in cardiovascular mortality of 18%, both highly statistically

significant. In the prevention trial 8% and 12% reductions in all-cause and cardiovascular

mortalities were seen and heart failure was prevented in 37% of patients (The SOLVD

investigators 1992).

A further trial, V-HeFT II, logically sought to compare the two vasodilator

regimens previously shown to be superior to placebo, namely enalapril or the combination

of hydralazine and isosorbide dinitrate (Cohn et al 1991). In spite of the fact that combination vasodilator therapy with hydralazine and isosorbide dinitrate resulted in a relatively greater improvment in left ventricular ejection fraction and exercise tolerance, enalapril treatment was associated with significantly greater reduction in mortality.

2.6.3.2 ACE inhibitors after myocardial infarction

The place of the ACE inhibitors in the treatment of all grades of chronic heart failure was established unequivocally from the results of the above studies. Studies in experimental animals (Pfeffer et al 1985) and also in humans (Pfeffer et al 1988, Sharpe et al 1988) have shown that the ACE inhibitors limit the progressive dilatation of the left ventricle which occurs after myocardial infarction. In addition the animal studies demonstrated increased survival after ACE inhibition (Pfeffer et al 1985). Given that the degree of left ventricular dilatation after myocardial infarction is a powerful prognostic indicator, the logical next step was to investigate the effect of the administration of an ACE inhibitor shortly after myocardial infarction. A number of trials have now been completed in this area.

In the CONSENSUS II study (Swedberg et al 1992), 6090 patients with acute myocardial infarction and presenting within 24 hours of the onset of chest pain were randomised to receive placebo or intravenous enalaprilat 1mg over 2 hours. Maintenance ACE inhibitor therapy was with enalapril 2.5mg twice daily increasing to 20mg once daily on day 5. No specific criteria pertaining to ejection fraction or severity of heart failure existed. The trial was terminated prematurely as determined by the study protocol when it became clear from interim analysis of the results that there was a high probability of no benefit from active therapy. Mortality was increased after 1 (7.2 % cf
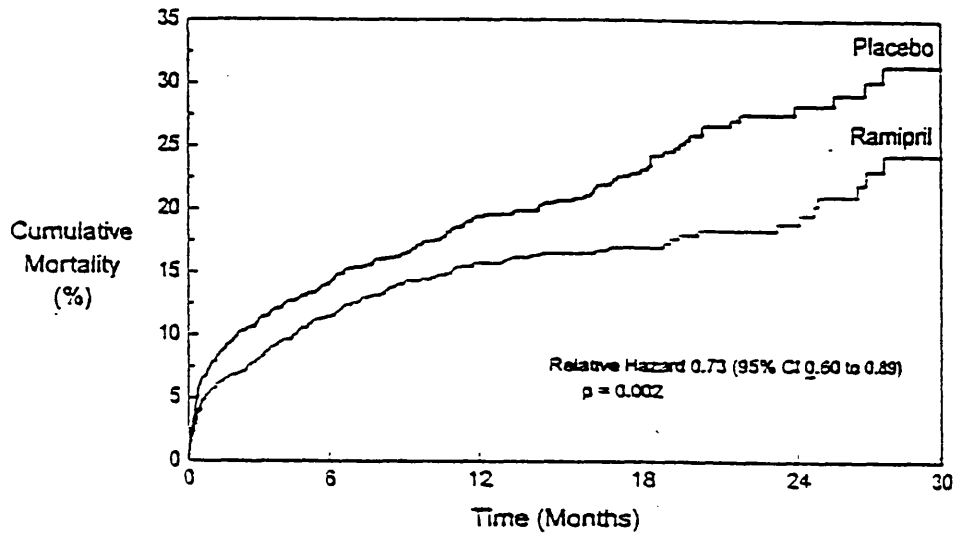
Figure 2.7.1:
Cumulative all-cause mortality in the ramipril and placebo groups in the
Acute Infarction Ramipril Efficacy (AIRE) study.

6.3 %) and 6 (11 % vs 10.2 %) months in the enalapril group compared to placebo and was higher in patients aged 70 years and over receiving enalapril. Development of hypotension after the first dose of ACE inhibitor was associated with increased mortality; 17 % of those patients developing first dose hypotension after enalapril died during follow-up compared to 9.3 % of those not showing this phenomenon. The study did however demonstrate a significant reduction in the incidence of the onset of heart failure in enalapril treated patients (27%) compared to placebo (30%).

In the Survival and Ventricular Enlargement (SAVE) trial 2231 patients were recruited within 3 to 16 days of myocardial infarction (Pfeffer et al 1992). Only those patients with ejection fraction of 40% or less, but without overt cardiac failure were randomised to receive placebo or captopril, 6.25mg as a test dose followed by 12.5mg titrated to a maximum of 50mg three times daily. Survivors were followed for 42 months on average. All-cause mortality was reduced from 25% in the placebo group to 20% in the captopril group, a risk reduction of 19%. Risk reduction for death from cardiovascular causes was 21%, for the development of severe heart failure 37% and for recurrent myocardial infarction 25%. These effects of captopril were independent of other demographic or treatment variables. It is perhaps of note that no difference in mortality between groups became apparent until 10 months of treatment.

The third study of the effects of ACE inhibitors on mortality and morbidity after myocardial infarction was the AIRE (Acute Infarction Ramipril Efficacy) study . In this study 2006 patients were randomised to receive placebo or ramipril 2.5mg daily, titrated up to 5mg or down to 1.25mg as tolerated. Recruitment was within 3 to 10 days of myocardial infarction and mean time of follow up was 15 months. Clinical evidence of heart failure was necessary for recruitment, although those with severe CHF or in whom

the open prescription of ACE inhibitor was felt clinically necessary were excluded. All-cause mortality was reduced from 23% for those receiving placebo to 17% for ramipril, an observed risk reduction of 27% (Figure 2.7.1). A risk reduction of 19% for death, severe heart failure, recurrent MI or stroke was observed (The Acute Infarction Ramipril Efficacy Study Investigators, 1993).

Most recently the TRACE (The Trandolapril Cardiac Evaluation) study demonstrated the efficacy of the new ACE inhibitor trandolapril in patients with echo-cardiographic evidence of left ventricular dysfunction after acute MI (Kober et al 1995). Patients were randomised to trandolapril or placebo between 3 and 7 days after infarction and followed up for between 24 and 50 months. There was a signficant 22 % reduction in mortality associated with active therapy and a 39 % reduction in progression to severe heart failure. There was no reduction in the incidence of recurrent myocardial infarction.

The overall results of these trials are positive with regard to the benefits which may be accrued from the use of ACE inhibitors soon after MI. The lack of benefit from enalapril in CONSENSUS II is somewhat surprising given the beneficial effects of enalapril upon left ventricular dilatation in man and additionally on mortality in experimental animals after myocardial infarction. However this study differs from the other two discussed above in terms of the very early and intravenous administration of the ACE inhibitor. The duration of follow up in this study was also much shorter than in the others. It is also of note that the timing of ACE inhibitor commencement in both AIRE and TRACE, ie, not before 3 days post MI, stemmed directly from the results of the CONSENSUS II study.

Although the results to date of the studies of the effect of ACE inhibitors after MI

Table 2.4

ACE inhibitors in congestive heart failure - clinical trials

TRIAL	TREATMENT	n	OUTCOME
CONSENSUS	Enalapril v placebo	253	27 % ↓ Mortality
SOLVD (Treatment)	Enalapril v placebo	2569	16 % ↓ Mortality; 26 % ↓ hospitalisations
V-HeFT II	Enalapril v hydralazine/ISDN	804	28 % ↓ Mortality (ACEI cf hydralazine/ISDN)
SOLVD (Prevention)	Enalapril v placebo	4228	37 % ↓ incidence of CHF; 36 % ↓ first hospitalisation
SOLVD (Combined)	Enalapril v placebo	6797	↓ Mortality ↓ Myocardial infarction ↓ Unstable angina ↓ Cardiac deaths

CONSENSUS=Cooperative North Scandinavian Enalapril Study
SAVE=Survival and Ventricular Enlargement
SOLVD=Studies of Left Ventricular Dysfunction
V-HeFT=Veterans Administration Cooperative Vasodilator Heart Failure Trial

Table 2.5 ACE inhibitors in heart failure after acute myocardial infarction - clinical trials

TRIAL	TREATMENT	n	OUTCOME (ACEI cf other treatment)
CONSENSUS II	enalapril v placebo	3046	No improvement in mortality
SAVE	captopril v placebo	2231	19 % ↓ mortality 37 % ↓ development severe CHF 25 % ↓ recurrent MI
AIRE	ramipril v placebo	2006	27 % ↓ mortality 19 % ↓ in first event after MI
GISSI-3	lisinopril v placebo	19394	11 % ↓ mortality 8 % ↓ combined end-points (death, CHF)
ISIS-4	captopril v ISMN v magnesium	58050	7 % ↓ mortality
TRACE	trandolapril v placebo	1749	22 % ↓ mortality 39 % ↓ development severe CHF
SMILE	zofenopril v placebo	1556	34% ↓ combined end-points (death, CHF)

AIRE=Acute Infarction Ramipril Efficacy; CONSENSUS=Cooperative North North Scandinavian Enalapril Study; GISSI=Gruppo Italiano per studio della Sopravvivenza nell'Infarto miocardico; ISIS=International Study of Infarct Survival; SAVE=Survival and Ventricular Enlargement; SMILE= Survival of Myocardial Infarction Long-term evaluation; TRACE=Trandolapril and Cardiac Evaluation.

indicate overall benefit, many questions remain unanswered. In particular it is unclear to which patients and at what stage in the development of heart disease physicians should be prescribing ACE inhibitors. Should their use be restricted to those with reduced ejection fraction ? Should an age limit be set ? What is the appropriate dose ? At least some of these questions may be answered by ongoing clinical trials.

2.6.3.3 The effect of ACE inhibition on mode of death in heart failure

As discussed above the role of the ACE inhibitors in the treatment of all grades of CHF and in heart failure following acute myocardial infarction has been demonstrated unequivocally. However the mechanism by which ACE inhibitors reduce the risk of death remains unclear. Some studies have ascribed the beneficial effect of ACE inhibition to a slowing of the progress of heart failure (Swedberg et al 1987, Yusuf 1991). A single study ascribed the benefit entirely to a reduction in the incidence of sudden death (Cohn et al 1991). Others have reported a benefit in terms of both reduction in heart failure progression and in sudden death (Pfeffer et al 1992, Kober et al 1995).

Ascribing a mode of death in individual cases is problematic and recent studies have highlighted the marked inter-observer variation that occurs in this respect (Ziesche et al 1995). Moreover the definition of death due to heart failure is controversial, as is that of a sudden death. There has been little uniformity of definitions among the various intervention trials of heart failure treatments and few studies have attempted prospectively to define what constitutes a death due to heart failure (Narang et al 1996).

The AIRE study included as an end point mode of death as defined by a predetermined classification system (Cleland et al 1993). This study also recorded place and cause of death, and also events associated with death. The details of this analysis have

recently been reported (Cleland et al 1997). Treatment with the active agent ramipril

reduced both progression to severe resistant heart failure by 23% and the risk of sudden

death by 30%. The incidence of death from circulatory failure was reduced by 18%

althought his difference did not reach statistical significance. Of interest is the finding that

over 80% of deaths classified as sudden were preceded by deterioration of the patient's

heart failure. The analysis provided no evidence to support the hypothesis that ACE

inhibition reduces the rate of reinfarction. The authors concluded that the main effect of

ACE inhibition on mortality in the AIRE study was through a reduction of progression of

heart failure. These findings are perhaps most important for highlighting the fact that most

sudden deaths occurring in patients with heart failure after myocardial infarction occur in

the context of worsening heart failure. There is no evidence to suggest that the same does

not hold for sudden death in heart failure in situations other than following myocardial

infarction.

CHAPTER 3

THE RENIN ANGIOTENSIN SYSTEM AND ANGIOTENSIN CONVERTING ENZYME INHIBITION

3.1 THE RENIN ANGIOTENSIN SYSTEM IN HEALTH AND DISEASE

The renin angiotensin system is ubiquitous in the vertebrate animal kingdom, and indeed may have a physiological role in some invertebrate species. To date it has been impossible to identify a unifying phylogenetic function for the system across all species. There is however no doubt as to its functional importance in man.

The importance of the renin-angiotensin system (RAS) in salt and water homeostasis was first noted by Tigerstedt and Bergman at the end of the last century. The RAS plays a pivotal role in cardiovascular homeostasis in terms of blood pressure control and salt and water homeostasis. The octapeptide angiotensin II is one of the most powerful vasoconstrictor agents known and is formed from its precursor angiotensin I by the action of angiotensin converting enzyme (ACE). Angiotensin I is itself formed from its precursor angiotensinogen under the influence of the protease enzyme renin. The activity of renin is rate limiting for the formation of angiotensin I. Recently, alternative pathways for the generation of angiotensin II have been demonstrated (de Silva et al 1988, Okamura et al 1990) but their physiological importance is as yet unclear.

3.1.1. Renin

At the time of the discovery by Tigerstedt and Bergman of a pressor substance extracted from the renal cortex of rabbits, its significance was not clear . However the characteristics of the substance, which they called "renin" were well defined at this stage

and were recognised to be those of a protein; intravenous administration elicited a pressor effect, the substance was destroyed by heating and did not pass through a semi-permeable membrane (Tigerstedt & Bergman 1898). Little research was published on the possible significance of these findings until the experiments over 30 years later of Goldblatt, who showed that clamping of the renal arteries produced sustained hypertension (Goldblatt et al 1934).

Renin was only purified in stable form in the 1970s. Its purification in large quantities from mouse submaxillary gland was described in 1982 (Misono et al 1982). Renin (EC 3.4.23.15) has a molecular weight of 38 kDa and belongs to the group of aspartyl proteases. Renin is initially produced from the gene as preprorenin, which undergoes post-translational processing to prorenin. The cleavage site of prorenin to renin is species specific; in man the activation of prorenin occurs at $-Arg_{66}-Leu_{67}-$. Renin has a number of characteristics which set it apart from other aspartyl protease enzymes. The enzyme is active at neutral pH, in contrast to the acid conditions favoured by other aspartyl proteases. The enzyme has marked substrate specifity, angiotensinogen being the only known substrate in vivo. However many body fluids contain acid proteases at much higher concentrations than that of renin, and these other enzymes can contribute a significant proportion of the renin-like activity of tissue extracts in vitro (Naruse et al 1981). Separation from other acid proteases is required for the accurate identification of renin in tissues. This may be achieved using fractionation on columns containing α-casein or haemoglobin or alternatively via the use of antibodies to renin. The use of renin antibodies has allowed the demonstration of the role of renin in blood pressure homeostasis in a variety of pathophysiological circumstances in a number of species, e.g., in spontaneously hypertensive rats (Inagami et al 1991), and salt-deplete dogs (Dzau et al

1980).

The renin-producing cells of the kidney are located in the juxtaglomerular apparatus (JGA). This consists of the distal afferent arteriole, the proximal efferent arteriole, the glomerulus, the macula densa (a specialised area of the distal tubule) and the Goormaghtigh cells, which lie in the area bordered by the macula densa and the two arteriolar portions. The function of the juxtaglomerular apparatus is thought to be the translation of renal tubular stimuli into changes in renin secretion and glomerular filtration rate. Renin producing cells are located in the media of the afferent arteriole where they replace the smooth muscle cells. The renin producing cells are contiguous with adjacent vascular smooth muscle cells, which are themselves capable of metaplastic transformation to renin-producing cells under conditions of chronic stimulation. Such conditions also stimulate the transformation of previously non-secretory JGA cells into ones containing renin granules. Such transformation occurs in response to stimuli which increase the release of renin and include: reduced renal blood flow; salt depletion; converting enzyme inhibition; beta-adrenergic stimulation; renal nerve activity. Acute activation of the system results in sodium retention secondary to increased aldosterone levels and to vasoconstriction secondary to increased angiotensin II concentrations. As might be expected, renin release is inhibited by angiotensin II.

3.1.2. Angiotensinogen

Angiotensinogen, or "renin substrate" is a 55-60 kDa glycoprotein present in plasma in micromolar concentrations. The human gene has been sequenced and located to chromosome 1q4. The genetic apparatus for the production of angiotensinogen is possessed by a variety of tissues and, while the angiotensinogen gene is expressed in a

number of tissues, expression within a particular organ is restricted to specific areas or cell types. Renal tubular cells in vitro are able to produce angiotensinogen (Yanagana et al 1991) and astrocytes appear to be the site of production in the CNS (Stornetta et al 1988). The presence is established in cardiac tissue of DNA, mRNA for angiotensinogen and angiotensinogen itself (Lindpainter et al 1990).

Changes in levels of angiotensinogen take place slowly in response to a number of stimuli. Glucocorticoids, oestrogens, angiotensin II and thyroid hormones stimulate the synthesis and release of angiotensinogen (Menard et al 1983). A low salt diet increases angiotensinogen mRNA in aortic smooth muscle cells (Naftalin et al 1991) and in renal proximal tubular cells while expression in the liver is unaffected (Ingelfinger et al 1990). Angiotensinogen may have a role in the inflammatory process. Elevated angiotensinogen levels are seen in patients suffering from a number of acute conditions such as pneumonia and acute pyelonephritis (Nielsen & Knudsen 1987). It has been postulated that angiotensinogen, or its metabolite des-Ang I-angiotensinogen, may act as an inhibitor of proteases, and that activation of local RAS in the presence of the vasodilation associated with fever may act to maintain blood pressure at a local level. Currently much interest is focused on the possible link between the angiotensinogen gene and hypertension.

3.1.3. Angiotensin peptides

3.1.3.1 Angiotensin I

Angiotensin I is an inactive decapeptide prohormone formed from the enzymatic activity of renin on angiotensinogen. In man angiotensin I is formed from the N-terminal portion of angiotensinogen by cleavage at Leu_{10}-Val_{11}. Angiotensin I is degraded to angiotensin II by cleavage of the N terminal dipeptide His-Leu, the reaction catalysed by

ACE.

3.1.3.2 Angiotensin II

Under physiological conditions, the conversion of angiotensin I to the active octapeptide angiotensin II occurs in significant amounts in the lung via the action of angiotensin converting enzyme located on the surface of pulmonary vascular endothelial cells. Due to the ubiquitous nature of ACE, formation of significant amounts of angiotensin II occurs across other vascular beds including those of the heart, kidney and spleen (Coghlan et al 1982, Campbell 1987, Oliver & Sciacca 1984).

Angiotensin II has a short half-life in vivo and is broken down by a variety of widely distributed angiotensinase enzymes. Catabolism occurs primarily in peripheral vascular beds. Angiotensin II is broken down into a number of fragments including the biologically active angiotensin III. Angiotensin III (des Asp_1-Ang II) retains approximately 30% of the pressor activity of angiotensin II. While in man concentrations are much lower than those of angiotensin II (Semple et al 1976) levels of angiotensin III in other species are relatively much higher. In man it is generally accepted that the physiological role of angiotensin III is minor. The peptide may have a role as a neurotransmitter in the CNS. A number of other peptide fragments of angiotensin II may be formed, particularly from the N-terminal of angiotensin II. Whereas in some animal species these appear to biologically active, their physiological relevance in man is unclear.

It is now apparent that a number of non-renin angiotensinogenases exist which are capable of generating angiotensin I and in some cases angiotensin II directly from angiotensinogen (Rosenthal et al 1990). In addition, a number of enzymes exist, in addition to ACE, capable of converting angiotensin I to angiotensin II (Okamura et al

1990). There is increasing evidence suggest that these pathways may be of physiological relevance. Firstly, such enzymatic activity can lead to generation of angiotensin II in sufficient quantities to elicit physiological responses. This is the case for isolated arteries (Bund et al 1989), isolated heart preparations (Hirakata et al 1990), and isolated perfused limb vascular beds (Ideishi et al 1990). The physiological relevance of these pathways is further suggested by the high substrate specificity for angiotensin I of some of these enzymes: the major angiotensin II forming enzyme in human heart extracts is a neutral serine protease with a high specificity for angiotensin I (Urata et al 1990). Finally, a number of these enzymes are located specifically in potentially important physiological sites (Okamura et al 1990).

The response of vascular smooth muscle cells to angiotensin II varies from transient contraction to hyperplasia and hypertrophy. The nature of the response is governed by the intracellular pathway activated by receptor activation, and the phenotypic state of the cell. Transient smooth muscle cell contraction results from increased intracellular Ca^{2+}, via phospholipase C mediated inositol triphosphate (IP_3) generation. The result is activation of myosin light chain kinase via calmodulin, with resultant phosphorylation of myosin, and actin-myosin cross-bridge formation. Angiotensin II also plays a role in sustaining smooth muscle cell contraction and stimulates the receptor operated calcium channels which are important in maintaining increased tone.

Angiotensin II stimulates hypertrophy of vascular smooth muscle cells in vitro (Geisterfer et al 1988). This response appears to be dependent upon gene transcription (Berk et al 1989). Hypertrophy occurs in response to prolonged stimulation with angiotensin II. While a generalised increase in protein synthesis is seen, a preferential increase in the synthesis of a number of specific proteins occurs, namely actin,

tropomyosin and vimentin. In some pathological conditions, angiotensin II causes vascular

smooth muscle cell hyperplasia. This is the case in some forms of hypertension (Paquet et

al 1990) and after balloon angioplasty (Daemen et al 1991). In this context the increase in

tyrosine phosphorylation (Tsuda et al 1991) and increase in proto-oncogene expression

(Naftilan 1992) which results from angiotensin II stimulation are likely to be relevant. The

increase in expression of platelet derived growth factor A-chain (PDGF-A), transforming

growth factor beta (TGF-β) and basic fibroblast growth factor (FGF) are all of relevance

to smooth muscle cell proliferation and in the context of chronic congestive heart failure

may be relevant to the remodelling process which is of such prognostic importance in this

condition. The relevance of the renin angiotensin system to vascular wall remodelling in

hypertension and myocardial remodelling in CHF is implied by the effect of the ACE

inhibitors in each condition (see Chapter 1). In hypertension, vascular smooth muscle cells

from hypertensive animals appear to be hyper-responsive to angiotensin II. Further to this,

angiotensin II elicits a relatively greater increase in the amount of extracellular matrix in

smooth muscle cells from the spontaneously hypertensive rat (SHR) compared to

normotensive animals (Scott-Burden et al 1991). In SHR, ACE inhibition with captopril is

more effective in inhibiting vascular hypertrophy than are other antihypertensive agents, in

spite of similar falls in blood pressure (Owens 1987).

3.1.3.3 Angiotensin II receptors

Specific angiotensin II receptors have been identified. The two major receptor

subtypes are designated AT1 and AT2. The former has been cloned and is selectively

blocked by biphenylimidazoles such as losartan (Dup 753). The AT2 receptor is blocked

by tetrahydro-imidazopyridines such as PD 123177.

The AT1 receptor is a 7 domain transmembrane G-protein which mediates smooth muscle cell contraction and aldosterone secretion as well as blood pressure and heart rate responses. The receptor is down-regulated in response to prolonged exposure to angiotensin II. The receptor appears to be coupled intracellularly to four enzymes, namely adenylate cyclase, phospholipase A2, phospholipase C and phospholipase D. Stimulation of AT1 receptors in hepatocytes, adrenal zona glomerulosa cells or pituitary cells results in a reduction in the activity of adenylate cyclase. The coupling of the AT1 receptor to phospholipase C (PLC) is likely to be of greater physiological significance. Receptor stimulation under these circumstances leads to the generation of the second messengers IP_3 and diacyl glycerol. This in turn activates cell contraction in the short term and cell hypertrophy and hyperplasia in the long term. In those cell types in which the AT1 receptor is coupled to PLC, there is also coupling to phospholipase D (PLD). This interaction may be important in cell growth.

While the physiological role of the AT2 receptor is unclear, evidence is accumulating for the existence of a physiological antagonism between the AT1 and AT2 receptors. Rogg and colleagues studied the relative numbers of angiotensin receptor subtypes in the atria of patients undergoing coronary artery or heart valve surgery, and the relationship between receptor subtype and a number of measures of cardiac function (Rogg et al 1996). Angiotensin II receptors were present in high density in membranes from human right atrium; AT1 receptors made up 33% of the population, AT2 receptors 67%. While there was no correlation between any measure of cardiac function and total receptor number or affinity, the proportion of AT1 receptors was higher in the atria of patients with normal right atrial pressure. Left ventricular ejection fraction was directly correlated with the proportion of AT1 receptors, while right atrial pressure was inversely

71

correlated with this parameter. The authors concluded that the ratio of AT1 to AT2 receptors correlates with left ventricular function and that the AT2 receptor is up-regulated in cardiac disease states. While AT1 receptor stimulation elicits cell proliferation and hypertrophy, an "anti-growth" effect of cardiac myocyte AT2 receptor stimulation has been demonstrated (Booz et al 1995). Thus, increased expression of the AT2 subtype in conditions of increased cardiac filling pressure and reduced ventricular function may be involved in the process of myocardial remodelling. On the basis of this and other evidence the suggestion has been made that there exists a functional antagonism between the AT1 and AT2 receptors (Dzau & Horiuchi 1996). This may have important implications for the physiological effects and therapeutic use of angiotensin II receptor antagonists: selective AT1 receptor antagonism may, by channeling angiotensin II to AT2 receptors, have important but as yet ill-defined effects on the remodelling process.

3.1.3.4 Assay of angiotensin peptides

The assay of angiotensin II and the other angiotensin peptides is based upon sensitive radioimmunoassay techniques (Nussberger et al 1988). The low picomolar concentrations of angiotensin II found in the plasma of man require a sensitive assay, particularly where levels are suppressed, eg by ACE inhibition. There is in addition a significant amount of cross-reactivity of the antibody with other angiotensin peptides such as angiotensin I. The very short plasma half-life of angiotensin II adds a further stumbling block to the accurate assay of angiotensin II levels. Thus sample collection into pre-chilled tubes containing a mixture of proteinase inhibitors is required, with rapid freezing of plasma. The in-vitro generation of angiotensin I, and the conversion of angiotensin I to angiotensin II must be prevented. Such a situation is particularly likely to pertain during

treatment with ACE inhibitors as a consequence of stimulation to renin secretion and high

angiotensin I levels. At the time of writing, the separation of angiotensin by reversed-

phase HPLC with subsequent radioimmunoassay is considered as state-of the art by

authorities in this field. Further to this, the estimation of the ratio of angiotensin II to

angiotensin I can be regarded as an estimate of in-vivo ACE activity during ACE inhibition

and may have advantages over in-vitro methods (Gorski et al 1991). The application of

this technique to the assessment of in-vivo activity of ACE is assessed as part of this

thesis.

3.1.4. Angiotensin converting enzyme

Angiotensin converting enzyme (ACE = kininase II = EC 3.4.15.1) was first

characterised in horse plasma (Skeggs et al 1956). ACE is known to be a very widely

distributed enzyme, present in plasma and in many tissues. The activity of the enzyme

varies markedly between species and also among tissues within a single species.

The bulk of angiotensin converting enzyme is membrane bound in vivo and activity

is found in the vascular endothelium of a number of tissues. ACE activity is higher in

arterial compared to venous endothelial cells. Epithelial cells tend to have greater ACE

activity than do endothelial cells: for example, the human kidney has 5-6 times more ACE

per unit weight than does the lung (Erdos 1990). High ACE activity is also found in other

heavily vascularised tissues such as brain and retina. ACE is also present in soluble form in

human plasma and is also found in other body fluids such as urine, semen, CSF and

amniotic fluid. Under physiological conditions in human plasma, angiotensin converting

enzyme is found in concentrations of approximately 400ng/ml although concentrations

vary according to genetic polymorphism for the ACE gene. With angiotensin I as

substrate, the enzyme has an absolute requirement for anions, in particular chloride. ACE contains zinc, which has an important role in substrate binding. A relative shortage of zinc may result in a reduction in activity of the enzyme.

ACE is a glycoprotein of molecular weight of approximately 147 000 Da. Membrane bound ACE is anchored by 17 amino acid region near the C-terminus. A single human gene encodes for the two types of membrane bound ACE, the endothelial form (~ 150 000 Da) and the testicular form (~ 90 Da). The shorter testicular form is produced via a tissue specific promoter. Its production is under the control of androgens, whereas production of the endothelial form appears more influenced by glucocorticoids. The process by which ACE appears in the plasma is poorly understood.

The endothelial enzyme is heavily glycosylated in vivo; human renal ACE contains approximately 25% by weight of a mixture of carbohydrates. The ratio of carbohydrate moieties varies in ACE from different tissues; while human lung ACE contains a majority of sialic acid residues, ACE from human kidney contains minimal amounts of sialic acid. The high proportion of sialic acid residues in lung ACE is in keeping with the putative origin of plasma ACE from this site, and appears to protect circulating ACE from uptake by hepatic lectins.

ACE contains two identical binding sites although it seems that only one active site is involved in enzyme activity under physiological conditions. Indeed the sites have different catalytic constants and patterns of chloride activation suggesting that they may each subserve different functions (Wei et al 1991). The enzymatic activity of the C-terminal domain is 3-10 times higher than that of the N-terminal domain. The activity of ACE is highly dependent upon chloride, although this requirement varies among substrates. While the conversion of angiotensin I to angiotensin II depends upon the

presence of chloride ions, the breakdown of bradykinin proceeds at approximately 30% of the maximal rate in the absence of chloride. Activation of ACE by chloride probably results from interaction of chloride with a lysine residue at or near the active site, resulting in a conformational change.

There appear to be at least four functional amino acid residues at or near the active site of ACE. Two histidine residues and one of glutamic acid coordinate the zinc atom at the active site while arginine binds the C-terminal carboxyl group of the substrate; a tyrosine molecule donates a proton to the amine moiety of the leaving group. The binding site for angiotensin I also binds ACE inhibitors. The zinc ion at the active site interacts with negatively charged elements of ACE inhibitor drugs. ACE is capable of catalysing the metabolism of a variety of peptide substrates, at least in vitro: angiotensin I, bradykinin, opioid peptides, substance P, neurotensin, luteinising hormone releasing hormone and gastrin. The relevance of this capability to the situation in vivo is not always clear.

Assay of angiotensin converting enzyme

A variety of laboratory methods exist for the assay of ACE. These largely involve either the use of a radiolabelled ACE inhibitor, or enzyme kinetic methods utilising one of a variety of natural or, more commonly, synthetic substrates for the enzyme.

Radiolabelled ACE inhibitors have been utilised in attempts to characterise tissue ACE, in particular with regard to putative differences in enzyme activity and drug penetration to the enzyme in different tissue sites. Marked differences in enzyme activity and affinity have been demonstrated; in the rat relatively low ACE activity is apparent in the kidney compared to the enzyme from lung (Jackson et al 1986). Autoradiographic

studies using tissue incubated with radiolabelled ACE inhibitor have localised high levels of ACE activity to the caudate nucleus, lung, renal cortex and adrenal medulla (Mendelsohn 1984).

Differences appear to exist among ACE inhibitors in terms of drug penetration to tissue ACE. For example perindopril inhibits renal and plasma ACE to a similar extent and with a similar time course, whereas inhibition of the enzyme from aorta and lung is more protracted but of a lesser degree (Jackson et al 1988). The relevance of such differences in man under physiological or pathophysiological conditions remains speculative.

The activity of ACE is routinely assessed using enzyme kinetic methods: these utilise as substrate either angiotensin I or, more commonly, any one of a number of synthetic polypeptides. The in vitro activity of ACE using angiotensin I as substrate is low. In addition, the presence of naturally occurring peptidases and the low sensitivity and specificity of the assay make the use of angiotensin I unsuitable for the routine assay of ACE activity. The spectrophotometric assay of hippuric acid or the spectrofluorometric assay of His-Leu generated from the tripeptide substrate Hip-His-Leu is one of the more commonly used assay methods (Cushman & Cheung 1971). Although this amino blocked tripeptide is resistant to the actions of aminopeptidases, the actions of His-Leu peptidases may lead to underestimation of ACE activity. Assay of ACE using this substrate is more sensitive than that using angiotensin I due to the greater activity of the enzyme with the synthetic substrate. A number of other amino-blocked synthetic tripeptide substrates are available for the assay of ACE activity. An additional number of intramolecularly quenched substrates have been synthesised . Assay of ACE activity using these substrates relies upon a change in the intensity of their fluorescence following cleavage by ACE. Finally, ACE activity can be assayed using hydrolysis of the carboxy terminal dipeptide of furanacrolyl-

Table 3.1 Synthetic substrates of angiotensin converting enzyme

N-acylated tripeptides:

> Hip-His-Leu
> Hip-Gly-Gly
> Bz-Phe-His-Leu

Furanacrolyl-blocked tripeptides:

> furanacrolyl-Phe-Phe-Arg
> furanacrolyl-Phe-Gly-Gly

Intramolecularly quenched tripeptides:

> ABz-Gly-Phe(NO_2)-Pro
> Bz(NO_2)-Gly-Trp-Gly

blocked tripeptides (Table 3.1).

3.1.5 Tissue based renin angiotensin systems

3.1.5. 1 Introduction

Until relatively recently the RAS was regarded as a purely circulating endocrine

system, the various components of which were secreted by a number of organs. As early

as 1964, the presence of renin in blood vessel walls was demonstrated (Gould et al 1964)

and it is now known that the genes for components of the RAS are found in a wide variety

of tissues (Dzau 1987). The organisation and physiological function of tissue based renin-

angiotensin systems remains unclear.

The most direct evidence that a significant amount of angiotensin I is generated

outside the intravascular compartment has come from studies of the metabolism of

angiotensin peptides across various vascular beds. A number of such studies have

demonstrated that within vascular beds, angiotensin I taken up and metabolised is replaced

by amounts of peptide generated within the tissue. Admiraal and colleagues demonstrated

extraction rates for angiotensin I of 47-96% in various vascular beds (Admiraal et al 1990,

Danser et al 1992, Danser et al 1992). In spite of these high extraction rates, similar levels

of angiotensin I were found in venous and arterial blood. These authors calculated that 20-

30% of angiotensin I activity from limb and kidney, and 60% of that from the gastro-

intestinal circulation is generated from circulating renin. The bulk of extravascular

angiotensin I generation appears to be due to renally derived renin. Indeed, angiotensin I is

almost undetectable in the plasma after nephrectomy.

The evidence for local synthesis of components of the renin angiotensin system is

overwhelming. Large amounts of renin are found in a variety of tissues, including areas of

the CNS outside the blood-brain barrier (Bunnemann et al 1993) and in other areas, such as testis (Inagami 1993). The high levels seen in these sites are most unlikely to have come from circulating renin. In a number of tissues renin is located at an intracellular site, favouring local synthesis. Renin synthesis has been observed in a number of cell culture lines including vascular smooth muscle (Re et al 1982) and endothelial cells (Tang et al 1990). Renin mRNA has been located in a variety of tissues other than the kidney including heart, brain and liver. A number of studies have demonstrated the presence of angiotensinogen in a similar variety of tissue sites.

The role of tissue-based renin angiotensin systems is unclear although a number of possibilities have been suggested. To date it has proved impossible to ascribe any single function to tissue based systems from different organs. As many components of tissue RAS are expressed in the foetus, a role in development is likely (Ingelfinger et al 1988). A role for tissue RASs in hypertension has been postulated. In a number of strains of genetically hypertensive rats the renin gene locus appears to be involved in hypertension in spite of normal circulating renin levels (Rapp et al 1989). In a hypertensive strain of transgenic rat incorporating a mouse renin gene, low levels of plasma renin and angiotensin II are seen in the presence of high renin gene expression in particular tissues, namely adrenal gland and vascular tissues (Mullins et al 1990). Of note in this model is that, compared to control rats without the mouse gene, there is increased tissue formation of angiotensin peptides (Hilgers et al 1992).

The blood pressure lowering effect of ACE inhibitors in normal and low renin conditions has been taken as evidence for a role of tissue RASs in blood pressure control, as has the dissociation between the effect of renin inhibitors on blood pressure and their effect on the circulating RAS. The ACE inhibitors were initially thought to exert their

physiological effects via inhibition of the enzyme in plasma. However there is a poor temporal correlation between the level of inhibition of plasma ACE and the haemodynamic effects of ACE inhibitors. This has been shown for a number of agents, for example captopril (Cohen & Kurz 1982), enalapril and lisinopril (Unger et al 1984). Although acute ACE inhibition results in a fall in plasma angiotensin II, during chronic ACE inhibition there occurs a reactive increase in renin with reemergence of angiotensin II generation (Mooser et al 1990). In addition, ACE inhibitors are effective in low renin states (Chatterjee & Opie 1987). Taken together this information suggests that the haemo-dynamic effect of ACE inhibitors may be due to inhibition of enzyme at a site other than in plasma, or alternatively to an effect other than inhibition of ACE. Of note is that in a number of animal models, such as the spontaneously hypertensive rat, the magnitude of the effect of ACE inhibition correlates better with inhibition of tissue ACE rather than the circulating enzyme (Unger et al 1984, Weishaar et al 1991).

3.1.5.2 Vascular tissues

Angiotensin converting enzyme is found on the luminal surface of the endothelial cells of all vascular beds. The physiological significance of ACE at this site in man is unclear. The enzyme has also been localised to the adventitia of the blood vessels of a number of species (Rogerson et al 1992) although in this context the enzyme is probably associated with the endothelial surface of the vasa vasorum.

Although all of the elements of the RAS have been demonstrated in the human vasculature, no definitive evidence of functional interaction of these elements yet exists. However the circumstantial evidence is strong. Campbell calculated that approximately 80% of the total venous angiotensin I and 70% of angiotensin II is from local production

(Campbell 1985). Similar figures have been calculated for the generation of angiotensin peptides in various vascular beds in man (Reams et al 1989, Schalekamp 1989). It has been suggested that locally produced angiotensin II may contribute to chronic hypertension and that circulating angiotensin II is of secondary importance to the autocrine and paracrine effects of that produced locally in terms of the regulation of vascular tone (Dzau 1988, Schalekamp et al 1989) and in blood pressure regulation (Dzau & Safar 1988).

As noted above the transgenic rat TGpos shows normal plasma renin activity in the presence of elevated vascular renin and angiotensin synthesis (Mullins et al 1990, Hilgers et al 1992). Although vascular renin activity in arterial tissue from rats with renovascular hypertension is elevated, this appears to be renally derived (Ubeda et al 1988). Elevated angiotensinogen mRNA synthesis has been reported in experimental hypertension (Shioto et al 1992). Vascular ACE activity is increased in a number of animal models of hypertension and is associated with increased mRNA for ACE (Shioto et al 1992). Although this evidence suggests that RAS components may have roles at a local tissue level, functional interaction of the components of the system as a whole has not been demonstrated. Alternative mechanisms have been suggested by which locally derived angiotensin II may influence vascular tone. A number of these involve interaction with the sympathetic nervous system at a local level. Indeed, there is little doubt that locally derived angiotensin II influences autonomic nervous control of vascular tone (for review see Squire & Reid 1993). The location of RAS components in the adventitial layers of the vasculature (Rogerson et al 1992) would of course be compatible with such a role.

There is also a possible role for local RAS in influencing the structure of both resistance and conduit arteries. Chronic administration of a low dose of angiotensin II

80

alters the structure of resistance arteries without affecting blood pressure (Griffin et al 1991). ACE inhibitors have in some studies been reported to better reverse vascular changes than other agents in the face of similar effects on blood pressure (Sano & Tarazi 1987). Further to this, ACE inhibition in the young spontaneously hypertensive rat results in a permanent reduction in blood pressure, an effect not seen in older animals (Harrap et al 1990). All of this evidence suggests a role for the RAS in the structural development of the small blood vessel. The RAS of larger blood vessels may also be important to the changes seen in pathological states such as hypertension. ACE inhibition improves large artery compliance in hypertensive subjects, an effect not seen in normotensive individuals (Simon et al 1985). No such improvement is seen with other antihypertensive treatments.

3.1.5.3 Cardiac tissues

While the evidence for the existence of a cardiac RAS is clear, its patho-physiological significance is unclear. Myocardial angiotensin II receptors have been identified in human ventricle (Urata et al 1989). These receptors have high affinity for the peptide, the Kd being around 1nM (Baker et al 1984). The second messenger system appears to be the inositol triphosphate/protein kinase C system. The result of receptor stimulation is mobilisation of intracellular free calcium, an effect which is augmented by stimulation by angiotensin II of L-type calcium channels (Dosemeci et al 1988). In cultured myocytes, angiotensin II increases the rate of spontaneous beating (Allen et al 1988). Angiotensin II also has a positive inotropic effect (Moravec et al 1990) and acts as a growth factor for myocytes (Schelling et al 1991). The isolated rat heart is capable of converting angiotensin I to angiotensin II, a reaction which can be prevented by ACE inhibition (Linz et al 1986). An alternative pathway to ACE for the production of

angiotensin II from angiotensin I exists in the human heart (Kinoshita et al 1991).

Thus activation of the RAS, for example in CHF, may theoretically result in a number of local effects of angiotensin II in cardiac tissues. As already noted, relative changes occur in the numbers of myocardial AT1 and AT2 receptors in cardiac failure (Rogg et al 1996). While activation is adaptive in the short term, long term activation of the RAS appears to be deleterious. Angiotensin II causes increased contractility of cardiac myocytes in vitro and has a constrictor effect on coronary arteries. Angiotensin II also elicits cardiac myocyte hypertrophy and interstitial collagen deposition in animal models of hypertension (Brilla et al 1990). In animal models, intravenous infusion of angiotensin II produces patchy myocardial necrosis (primarily in the left ventricle) in a concentration related manner (Kremer et al 1981). Modulation of the RAS in such circumstances may therefore have important therapeutic implications for the heart (Table 3.2). As discussed previously, a physiological antagonism may exist between the cardiac AT1 and AT2 angiotensin II receptors (Dzau & Horiuchi 1996).

Although the above evidence suggests a possible role for the RAS in terms of structural and functional changes occuring in the myocardium in disease processes it is not clear to what extent the cardiac, as opposed to the circulating RAS, is specifically involved. The right side of the heart, in particular the right atrium, is the main cardiac site for angiotensin II production and ACE activity (Johnson et al 1989, Rosenthal et al 1987). High concentrations of ACE activity are found on the surface of the valves and especially in association with the coronary vasculature (Fabris et al 1989). Specific induction of cardiac ACE activity has been demonstrated in animal models of experimental heart failure (Hirsch et al 1991); ACE activity was not induced in other tissues or in plasma. It may be of significance that in these studies, there was a positive correlation

Table 3.2 Possible therapeutic implications of cardiac ACE inhibition

1. Myocardial hypertrophy:
    - reduced left ventricular hypertrophy in hypertension
    - normalised isomyosin profile

2. Congestive heart failure:
    - improved mortality/morbidity compred to alternative vasodulator regimes
    - activation of cardiac mRNA and angiotensin II in experimental heart failure

3. Ischaemic heart disease
    - reduction of post ischaemia arrhythmias by ACE inhibitors
    - benefit antagonised by angiotensin II and by bradykinin

4. Post infarct remodelling
    - Improved experimental remodelling by ACE inhibitors   -

between the level of right ventricular ACE activity and the size of experimentally induced myocardial infarction. The size of myocardial infarction correlates well with the degree of impairment of left ventricular function (Pfeffer et al 1979). By implication the magnitude of activation of the cardiac RAS is related to the degree of left ventricular dysfunction. Such findings may be relevant to the beneficial effects of ACE inhibitors in heart failure. Treatment with ACE inhibitors does reduce infarct size (Ertl et al 1982) and ventricular remodelling (Pfeffer et al 1988, 1992; SOLVD 1992) in both experimental and clinical heart failure. Similarly, ACE inhibition reduces the pathological structural changes which occur secondary to ventricular hypertrophy (Michel et al 1988, Yoshimura et al 1989). In vitro studies have confirmed that cardiac ACE shows differing binding affinities for various inhibitors (Fabris et al 1989, vide infra). Thus the potential exists for differential effects among ACE inhibitors in terms of inhibition of cardiac ACE.

3.1.5.4 Pulmonary tissues

The pulmonary vasculature has long been regarded as the major site of conversion of angiotensin I to angiotensin II (Ng & Vane 1968, Said 1982). As in other tissues, ACE activity in the lung is located largely on the endothelial surface of the blood vessels. The enzyme is inducible, as occurs with chronic ACE inhibitor therapy (Fyhrquist et al 1982) and an increase in plasma ACE activity has been regarded as a marker of lung injury in a number of settings (Foresti et al 1989, Kelley et al 1988, Oliver et al 1989). Apparent inhibition of pulmonary ACE has been demonstrated after administration of ACE inhibitor. In such studies the time course of the hypotensive response to the ACE inhibitor correlated better with pulmonary as opposed to plasma ACE inhibition (Chen et al 1984). More prolonged inhibition of pulmonary ACE has been demonstrated following ramipril

compared to enalapril (Unger et al 1985). In the only study to date of the trans-pulmonary kinetics of an ACE inhibitor, MacFadyen and colleagues attempted to define the trans-pulmonary uptake of perindoprilat given by intravenous infusion in patients undergoing cardiac catheterisation (MacFadyen et al 1991). Uptake into tissue sites would be expected to alter the plasma concentration time profile. In spite of the high concentration of ACE in lung tissue this study failed to demonstrate any significant transpulmonary uptake of drug. The significance of this finding is unclear.

3.1.5.5 Brain tissues

The presence of components of the RAS in the CNS has long been recognised although their precise functional significance is not clear. A facilitatory interaction exists between the RAS and the sympathetic nervous system: angiotensin II appears to enhance the effect of adrenergic neurotransmission at a number of sites (for review see Squire & Reid 1993). Most areas of the CNS are within the blood-brain barrier and as such are not readily accessible to either angiotensin II or to ACE inhibitors. A number of areas of the CNS outside the blood-brain barrier, in particular the circumventricular organs, do possess ACE activity and their proximity to structures such as the dorsal motor nucleus of the vagus nerve make these areas well placed to modulate autonomic function. Angiotensin II has a central pressor action, as shown in the landmark experiments of Bickerton and Buckley (Bickerton and Buckley 1961). This effect is mediated via increased central sympathetic outflow. Direct intra-cerebroventricular injection of angiotensin II elicits a pressor response and drinking via actions at distinct sites. Circulating angiotensin appears to play a role in the release of a number of pituitary hormones, in particular vasopressin and prolactin. All components of the RAS, including angiotensin II receptors, have been

demonstrated in distinct areas of the CNS. The presence of mRNA for angiotensinogen (Dzau et al 1986) and the localisation of its expression to the astrocytes (Stornetta et al 1988) has confirmed the presence of an endogenous brain RAS. A particularly high degree of correlation between the various components of the RAS is seen in the hypothalamus. Thus the possible roles for brain renin angiotensin systems include blood pressure and body-fluid homeostasis, regulation of pituitary hormone release, and perhaps also as a local neurotransmitter.

ACE inhibitors given locally into the cerebral ventricles cause a hypotensive response in experimental hypertension (Sakaguchi et al 1988). The penetration of ACE inhibitors to areas outside the blood-brain barrier is concentration related and in comparative studies, some agents do not penetrate in the administered doses (Sakaguchi et al 1988). The relative pharmacokinetic properties of different agents may be relevant in this respect. However there is no conclusive evidence for a central action of ACE inhibitors in any pathophysiological condition.

3.1.5.6 Renal tissues

Given the importance of the kidney in salt and water homeostasis it is not surprising that the renal RAS has a vital role in the normal functioning of this organ. In phylogenetic terms the renal RAS appears to predate the appearance of species with lung tissue. Although the physiological role of this system is unclear it has been suggested that the intrarenal RAS represents a primitive volume-control apparatus. In addition to the endocrine function of the kidney in releasing renin in response to stimuli such as salt depletion, the autocrine functions of renal based elements of the RAS are now increasingly seen of importance. All the elements of the RAS are known to be present in

renal tissue. The renal content of mRNA for angiotensinogen is increased by low-salt diet, providing a mechanism for activation of the local RAS in response to this stimulus (Ingelfinger et al 1986). ACE activity is found not only in association with the renal vasculature but also with the tubular and interstitial tissues (Marchetti et al 1987). Although angiotensin I is converted to angiotensin II in the kidney, local production means that there is little arteriovenous gradient in angiotensin I (Admiraal et al 1990). The cells of the brush border of the proximal tubule contain high levels of ACE, and the fluid of the proximal tubules contains concentrations of angiotensin II many times higher than seen in plasma (Siekely et al 1990). A possible role in the control of proximal tubule sodium reabsorption has been suggested for the RAS in this location (Ingelfinger et al 1990). Renin secretion from the juxtaglomerular cells is under the influence of a number of local factors including renal baroreceptor input, salt delivery to the macula densa, angiotensin II, and atrial natriuretic factor (Davis & Freeman 1976). The final response is dependent upon integration of the neural and hormonal input.

Angiotensin II has multiple roles in the kidney, all of which lead to salt and water conservation. As noted above, all the elements of the RAS have been localised to renal tissue, and it is generally accepted that angiotensin II is generated within renal tissue. Angiotensin II acts on both afferent and efferent arterioles to modulate pre- and post-glomerular resistance with preferential activity at the efferent side of the glomerular microcirculation. Angiotensin II also elicits directly contraction of the mesangial cells, resulting in reduction of the available filtration area and thereby of the glomerular ultrafiltration coefficient. Subpressor doses of angiotensin II elicit marked reduction in urine flow and sodium excretion, independent of changes in systemic and renal haemodynamics (Levens et al 1981), suggesting an action on the renal tubule. Auto-

radiographic studies have localised angiotensin II receptors primarily to renal cortical glomeruli and secondarily to the outer cortex. The former are down-regulated by salt depletion and by angiotensin II, and up-regulated by ACE inhibition. The latter receptors, located in the area of the proximal convoluted tubule, are up-regulated by sodium depletion and down-regulated by salt loading and by guanine nucleotides. Such distribution and properties suggest multiple intrarenal actions of angiotensin II.

The renal RAS may have a role in the development of hypertension in genetically hypertensive rats. In young WKY rats before the development of hypertension, high renal mRNA for renin, renin levels, angiotensinogen levels and angiotensin II concentrations have been reported. However plasma renin levels are not elevated (Samani et al 1989). All these parameters are normal in older WKY rats and it has been suggested that the overactivity of the renal RAS in the young animals is the trigger to the development of hypertension (Harrap 1991).

Renal artery stenosis leads to hypertension via an increase in peripheral vascular resistance. While in man renin is likely to be the major pathogenetic factor, plasma renin levels are elevated in only 50% of patients with renovascular hypertension (Grim et al 1977). However there is a positive correlation between blood pressure and plasma angiotensin II levels (Brown et al 1979) and increased sensitivity to angiotensin II exists in the chronic phase of renovascular hypertension.

3.1.6 ACE inhibition and renal artery stenosis

Glomerular filtration depends upon differential tone between the afferent and efferent glomerular arterioles (Edwards 1983). The tone of these vessels is under the control of angiotensin II, the effect of receptor stimulation being relatively greater on the

efferent vessel. Efferent arteriolar tone is exquisitely dependent upon angiotensin II in situations of low perfusion pressure (Blythe 1983). The classical example of this is in renal artery stenosis, although such conditions may often exist in situations of sodium depletion and in CHF. In early case reports of reversible renal failure in patients with reno-vascular hypertension treated with ACE inhibitors (Farrow & Wilkinson 1979), it was unclear as to whether the treatment itself or the fall in blood pressure was to blame.

While the anatomical effects of stenosis of the renal artery, via clipping in experimental animals, are clear, the relevance of these changes to the situation in man are less so. This largely arises from our uncertainty about the natural history of the condition in man. However there is little doubt that ACE inhibition in such patients results in impairment of the function in the affected kidney or kidneys. In patients with unilateral renal artery stenosis ACE inhibition results in a fall in glomerular filtration rate (GFR) (Miyamori et al 1986) and creatinine clearance (Ribstein et al 1988). Compensatory increases occur in GFR and effective renal plasma flow in the contralateral kidney (Miyamori et al 1988). These changes are to some extent independent of the degree of blood pressure fall (Miyamori et al 1988). However any treatment which elicits a fall in systemic blood pressure may cause a fall in GFR in the presence of renal artery stenosis (Textor et al 1985).

The propensity for ACE inhibitors to elicit this effect in the presence of renal artery stenosis, even in the absence of a fall in blood pressure, is a result of intrarenal effects, and is the basis of the diagnostic use of the captopril renogram (Geyskes et al 1987). In such patients the fall in blood pressure after ACE inhibition predicts the blood pressure response to surgical correction of the lesion (Staessen et al 1988). The reduction in renal

function which accompanies brief ACE inhibition is reversible; a similar approach is used in commencing therapeutic ACE inhibition, in which renal function is routinely monitored during the few days after initiation of therapy.

Untreated renal artery stenosis is associated with renal artery occlusion from a variety of causes. The use of ACE inhibitors has been associated with an increased incidence of this phenomenon (Hollenberg 1983). The evidence for such an association is however limited. The presence of sodium depletion adds to the risk of deteriorating renal function with ACE inhibition, and significantly to the occurrence of renal artery occlusion (Postma et al 1989). A study of the efficacy of enalapril plus hydrochloro- thiazide therapy compared to a regimen of timolol, hydralazine and hydrochloro- thiazide in the treatment of renovascular hypertension showed a relatively greater fall in systolic blood pressure with the regime containing the ACE inhibitor (Franklin & Smith 1985). This was at the expense of deterioration in renal function in 10 of 49 patients treated with enalapril compared to 1 of 39 treated with the alternative regime. Similarly, reduction in renal function in patients with renovascular disease is more likely to occur in those treated with a combination of ACE inhibitor plus diuretic (Hollenberg 1983).

Thus the routine treatment of renovascular hypertension with a regime containing an ACE inhibitor cannot be recommended. Similarly, the use of ACE inhibition in patients with CHF must be cautious where renovascular disease is suspected. At-risk patients will include those with a history of severe hypertension, and widespread arterial disease including peripheral vascular disease (Salmon & Brown 1990). However some authorities suggest that a trial of ACE inhibition may be prudent in patients with severe hypertension (Hollenberg 1993), particularly in the elderly in whom the risk from uncontrolled

hypertension is substantial.

3.1.7 Diabetic nephropathy

A number of disturbances of the RAS occur in diabetes mellitus, both type I, insulin dependent (IDDM), and type II, non-insulin dependent diabetes (NIDDM). No abnormalities are seen during the first few uncomplicated years of insulin-dependent diabetes. Thereafter the conversion of prorenin to renin appears to be impaired, the abnormality worsening as diabetic nephropathy progresses in severity (Wilson & Leutscher 1990). In patients with diabetic nephropathy plasma prorenin levels progressively increase through the stages of intermittent proteinuria, persistent proteinuria and overt nephropathy (Leutscher & Kraemer 1988) and correlate with the urinary albumin/ creatinine ratio. While prorenin levels are often elevated, plasma renin levels are normal or low.

In diabetes the pressor response to angiotensin II is increased and the renal response to angiotensin II, in terms of the usual reduction in GFR and renal plasma flow, is blunted (Reineck & Kreisberg1983). Angiotensin II however enhances filtration fraction in diabetes (Bank et al 1988). Thus the loss of normal renal haemodynamic control by angiotensin II may play a part in the development of the hyperfiltration which is a feature of diabetic nephropathy. Similar changes in the components and behaviour of the RAS are seen in diabetes complicated by retinopathy or neuropathy, and are also seen in patients with non-insulin dependent diabetes. In both experimental and human diabetes, diabetic microangioapathy affects glomeruli and arterioles including the juxtaglomerular apparatus, with separation of the renin secreting cells from the macula densa. In end-stage diabetic renal disease, there are significantly fewer renin secreting cells, as would be expected in a

situation of hyperfiltration of the renal microcirculation. It is not clear if the renal histological changes are linked to the observed abnormalities of circulating renin and prorenin.

The importance of proteinuria in diabetes is in its relationship to prognosis. In prospective studies in IDDM patients the presence of persistent microalbuminuria is a sensitive but non-specific marker of patients at risk of developing diabetic nephropathy. In the individual patient the specificity for predicting nephropathy can be improved by considering other factors, namely blood pressure and the degree of metabolic control. In patients with NIDDM the presence of microalbuminuria is a strong predictor of mortality. The majority of the excess mortality seen is from cardiovascular disease (Mogensen 1984); indeed microalbuminuria is more strongly associated with cardiovascular death in NIDDM than with end-stage renal disease (Schmitz et al 1990).

A number of therapeutic strategies slow the rate of progression of diabetic nephropathy. These are protein restriction (Zeller et al 1991), optimal glycaemic control (KROC collaborative study group 1984) and the control of hypertension (Mogensen 1982). To date only the control of hypertension has been associated with improved mortality in addition to a reduction in the rate of deterioration of renal function (Parving et al 1987).

ACE inhibition in diabetic nephropathy: data from animal models

Data from experimental models of diabetes suggest that increased glomerular capillary pressure is important in the development of renal injury in diabetes (Hostetter et al 1982). Crucially, increased glomerular capillary pressure occurs in the absence of systemic hypertension, and renal renin appears to be involved in mediating the increase

(Anderson et al 1990).

Studies in animals suggest ACE inhibition may provide benefits to diabetic patients over and above simple blood pressure control. In the normotensive, hyperglycaemic rat, enalapril reduces glomerular capillary pressure with no effect on glomerular filtration (Zatz et al 1986) and with concomitant reduction in albuminuria (Zatz et al 1987). Further to this, while the combination of hydralazine, reserpine and hydrochlorothiazide slows the development of proteinuria in diabetic rats, captopril treatment results in a similar blood pressure and the prevention of nephropathy (Anderson et al 1989). In this study the early fall in glomerular pressure seen with combination therapy was not sustained, and in spite of a sustained effect on blood pressure, glomerular hypertension developed late. In the obese Zucker rat, metabolic disturbances akin to those seen in NIDDM are seen. These animals show moderate hyperglycaemia, insulin resistance, hyperlipidaemia, obesity and hypertension from around 10 weeks of age. Elevation of blood presure is accompanied by progressive glomerular injury. In this model of NIDDM, enalapril reduced blood pressure, albuminuria and renal glomerular injury compared to control rats (O'Donnell et al 1989). The lack of effect of enalapril on glomerular pressure in this model may suggest that ACE inhibition may be renoprotective by mechanisms other than via reduced glomerular pressure.

ACE inhibition in diabetic nephropathy: data from human studies

Data regarding the effects of ACE inhibition on proteinuria in the early stages of diabetic nephropathy in man are conflicting. In one study ACE inhibition resulted in a reduction in urinary albumin excretion (Pedersen et al 1988). Other studies have failed to confirm this (Pasa et al 1987, Brichard et al 1989). In IDDM patients with incipient

proteinuria, a number of studies have demonstrated that urinary protein excretion is reduced by ACE inhibition. The beneficial effect is seen irrespective of the presence of systemic hypertension. In normotensive patients, progression of microalbuminuria to overt nephropathy was prevented by enalapril, which reduced fractional excretion of albumin and prevented the fall in GFR which was seen in patients receiving placebo over a one year period (Marre et al 1988). Data from longer term studies suggests that the effect is sustained. In normotensive IDDM patients, captopril prevents the progression of microalbuminuria to overt nephropathy, i.e. albuminuria > 300mg/24 hr (Mathiesen et al 1991). A similar effect is seen in hypertensive IDDM patients and in those with type II diabetes (NIDDM) (Melbourne Diabetic Nephropathy Study Group 1991). In patients with advanced diabetic nephropathy (renal impairment, marked proteinuria and hypertension), ACE inhibition reduces urinary protein excretion to a similar extent to that seen in less severe disease (Parving et al 1988).

A number of studies comparing ACE inhibition with alternative anti-hypertensive therapy, primarily calcium channel blockers, have been conducted. In the Melbourne study, nifedipine had no effect on albuminuria in normotensive diabetic patients in contrast to the reduction seen with the ACE inhibitor perindopril, although this was not statistically significant. In hypertensive patients in this study, both drugs were associated with similar reductions in albuminuria, as was the case in a comparison of enalapril and nicardipine in hypertensive NIDDM patients (Baba et al 1989). Other studies suggest a comparatively better effect of ACE inhibitor compared to calcium anatagonist in normotensive IDDM patients with incipient nephropathy (Insua et al 1988). In advanced diabetic nephropathy ACE inhibition with enalapril produced a greater reduction in albuminuria than β-blockade with metoprolol in spite of similar blood pressure falls (Bjorck et al 1990). However

similar effects on albumin excretion have been seen in comparisons of lisinopril and diltiazem (Bakris 1990), captopril and nicardipine (Stornello et al 1989) and enalapril and atenolol (Stornello et al 1991). Angiotensin converting enzyme inhibition slows the progression of advanced diabetic nephropathy (Bjorck et al 1986), although the rate of loss may be similar with β- blockade (Bjorck et al 1990).

Thus ACE inhibition reduces urinary protein excretion in all stages of diabetic nephropathy. The extent of the reduction in proteinuria correlates with the fall in blood pressure. In a multivariate analysis of 24 trials of ACE inhibitor treatment in diabetic nephropathy, Kalil et al (Kalil et al 1993) calculated that the fall in blood pressure could explain only 12.4% of the reduction in protein excretion. These authors postulated that intrarenal mechanisms, among others, may play a role in the response.

3.1.8 The renin angiotensin system in CHF

Activation of the RAS in the condition of congestive heart failure was first described in 1946 (Merrill et al 1946), but largely forgotten or ignored for 20 years thereafter. The significance of the RAS in CHF was confirmed by the effects of inhibitors of angiotensin II (Johnson & Davis 1973), ACE inhibitors (Freeman et al 1979), and renin inhibitors (Fitzpatrick et al 1990) on haemodynamic and neuro- hormonal parameters in the condition. At the onset of left ventricular dysfunction, plasma renin activity and aldosterone levels rise. As a new steady state is reached, plasma renin activity returns towards baseline values. The rise in plasma renin activity may be in part inhibited by elevated atrial natriuretic factor levels (Lee et al 1989). Thus renin levels are often normal in stable CHF (Francis et al 1990). Acute heart failure is associated with activation of the RAS and concomitantly high plasma renin levels (Dzau et al 1981). The aetiology of CHF

does not appear to influence the magnitude of the response, although the degree of activation of the RAS has been suggested to be lower in high-output cardiac failure due to beri-beri than in low output conditions (Ikram et al 1981).

Strong inverse relationships between plasma renin levels and arterial blood pressure on the one hand and fractional excretion of sodium on the other suggest that the afferent arteriolar baroreceptor and macula densa are important in regulating renin secretion in CHF. Renal sympathetic nerve activity is increased in CHF in man and there is good correlation between plasma renin and catecholamine levels well. This suggests that increased renal sympathetic activity contributes to elevated plasma renin activity, and is in keeping with information from animal studies. Cardiac and arterial baroreceptor dysfunction may contribute to increased sympathetic activity. Elevated angiotensin II and concomitantly elevated aldosterone levels contribute to elevated total body sodium and reduced total body potassium in CHF. In addition there appears to be increased renal responsiveness to aldosterone (Barger et al 1959).

While diuretics remain the mainstay of the treatment of CHF, such therapy alone does not improve prognosis. Indeed, by reducing circulating fluid volume, diuretics can reduce ventricular filling pressure and contribute to both the activation of the RAS and the electrolyte disturbances seen in CHF. In the patient with mild CHF in whom there is little or no salt and water overload, diuretic treatment stimulates a rise in plasma renin activity. This contrasts with the lack of such effect with acute treatment in oedematous patients, in whom plasma renin may initially fall in response to natriuresis before rising as natriuresis diminishes (Brown et al 1970). Chronic diuretic therapy results in elevated plasma renin activity. In the long-term there is a correlation between maintenance diuretic dose and plasma renin and angiotensin II levels (Fitzpatrick et al 1985). The addition of a potassium

sparing diuretic to chronic loop diuretic treatment elicits a brisk activation of the RAS

(Nicholls et al 1976).

The reported effect of vasodilators on plasma renin activity in CHF is variable.

Short-term infusion of nitroglycerin has been reported to have no effect (Packer et al

1987b) or to elicit a moderate rise in plasma renin activity (Dupuis et al 1990). The

available data for calcium channel blockers is likewise inconclusive. Plasma renin levels are

stimulated by intravenous administration of digoxin (Covit et al 1983) while there is no

effect of chronic oral therapy (Alicandri et al 1987). While dobutamine elicits a rise in

plasma renin activity (Uretsky et al 1986), no effect is seen with dopamine (Maskin et al

1985).

There is little doubt that the RAS is of fundamental importance in the patho-

physiology of human CHF. Indeed plasma renin activity and activation of the RAS as a

whole is one indicator of prognosis in CHF (Packer et al 1987a). The evidence for a

central role for the RAS in the pathophysiology of CHF is compelling. In addition to a role

in altered central and peripheral haemodynamics, the RAS has profound influence on salt

and water homeostasis. Direct vasoconstrictor effects of angiotensin II play a central role

in the haemodynamic changes of CHF. Correlations have been reported between a variety

of haemodynamic variables, such as cardiac output, systemic vascular resistance and

ventricular filling pressures, and plasma renin activity. The haemodynamic response to

ACE inhibition and to angiotensin II receptor antagonism (Cody et al 1982) has been

related to pre-existing activity of the RAS. The relatively weak relationship between pre-

treatment RAS activity and the haemodynamic response to ACE inhibition is one factor

which has led to the suggestion that the RAS at one or more tissue sites may be more

important than the circulating system in mediating the acute blood pressure response

(Dzau 1988).

The correlation between plasma renin activity and ventricular ectopic activity, and the reduction in such activity seen with ACE inhibitor treatment in CHF suggest a potential pro-arrhythmic effect of an activated RAS. Finally it is important to note that the degree of activation of the circulating RAS correlates inversely with prognosis in CHF (Rockman et al 1989). In this respect the relative role of RAS in renal and other tissues is as yet unclear.

3.1.9 ACE inhibition in CHF

3.1.9.1 Haemodynamic effects

The haemodynamic effects resulting from the administration of ACE inhibitor therapy in patients with CHF are well described and consistent. Arterial blood pressure and peripheral vascular resistance fall rapidly by around 20-35%. Cardiac output rises by 10-20%, largely due to an increase in stroke volume. Left ventricular filling pressure and pulmonary capillary wedge pressure, usually elevated in CHF, fall by about 40%. Regional circulatory changes vary among vascular beds: renal vascular resistance shows the largest falls, with lesser changes in the coronary, cerebral and skeletal muscle vascular beds (Creager et al 1981, Crozier et al 1989). It is important to note that for patients with stable CHF, the haemodynamic response to ACE inhibition is maintained with long-term therapy (Packer et al 1983). Systemic venous dilatation may be of particular benefit to patients with CHF. The characteristic lack of reflex tachycardia following vasodilatation with ACE inhibition may be accounted for by the so-called "parasympathomimetic" effects of these drugs (Campbell et al 1985), although inhibition of the synergy between the RAS and sympathetic nervous systems may be relevant (Squire & Reid 1993).

3.1.9.2 First-dose hypotension

In spite of the unequivocal evidence of the beneficial effect of ACE inhibition in CHF, a significant proportion of patients who may benefit from treatment are not prescribed an ACE inhibitor (Clarke et al 1994). Failure to prescribe this most appropriate of therapies stems at least in part from physicians' perceptions of two potential adverse effects of initiation of ACE inhibitor treatment, renal impairment and first-dose hypotension.

The relative magnitude of the initial blood pressure response to ACE inhibition is greater in CHF than in patients with hypertension (Wenting et al 1982). The incidence of first-dose hypotension varies according to patient populations and reporting procedures, some studies suggesting 10-15% (Hodsman et al 1983), others 30-40% (Packer et al 1986). In patients with heart failure the incidence of *symptomatic* hypotension is approximately 5-10% (The AIRE Study Investigators 1993, The CONSENSUS Trial Study Group 1987); 2-5% of patients withdraw from treatment as a result. In one of the early intervention trials of ACE inhibitors in heart failure, the occurrence of first dose hypotension was considered a sufficient problem to warrant a reduction in the starting dose of enalapril (The CONSENSUS Trial Study Group 1987). In CONSENSUS II, mortality was higher among those patients who developed first-dose hypotension after initiation of therapy with enalapril compared to those who did not (Swedberg et al 1992). The incidence of significant first-dose hypotension appears to have fallen as ACE inhibition has been introduced much earlier in the course of CHF.

Concerns regarding first-dose hypotension relate to the risk of renal, myocardial or cerebral hypoperfusion which may result (Cleland et al 1985) and have led to the development of a number of anecdotal protocols for the introduction of ACE inhibitor

therapy in CHF in an attempt to minimise the likelihood or duration of hypotension (McMurray et al 1989). Such protocols have often involved admission of patients to hospital for a number of days, entailing inconvenience to the patient and having resource implications for the physician and institution. A number of case reports have highlighted the potential problem (Mujais et al 1984, Cleland et al 1985).

A number of physiological and biochemical parameters have been suggested to identify patients at high risk of significant first-dose hypotension. Elderly patients (O'Neill et al 1988) and those with pre-treatment activation of the RAS (Cleland & Oakley 1991) are thought to be at relatively high risk. However the magnitude of the relationship between haemodynamic response and plasma renin activity at baseline is weak (Packer et al 1985) and in some well-conducted, prospective studies not apparent (Motwani et al 1994). The long-term blood pressure response to ACE inhibition is even less well related to pre-treatment plasma renin activity (Packer et al 1985). It has been suggested that the determinants of the response to chronic ACE inhibition may differ from those determining the acute response (Cleland & Dargie 1987). However although the phenomenon of symptomatic hypotension after ACE inhibition is best recognised as occurring at initiation of therapy, the available evidence indicates that the blood pressure response to the first dose of ACE inhibitor is the same as that seen after many months of treatment in patients with CHF (McLay et al 1992, Packer et al 1986b).

A number of factors, which have in common their ability to increase angiotensin II levels, have been suggested to increase the risk of first-dose hypo- tension. These are low pre-treatment blood pressure, high diuretic dose, hypo- natraemia and renal artery stenosis (Cleland & Oakley 1991). In patients with more severe grades of CHF, hyponatraemia and hypovolaemia associated with high doses of loop diuretic have been recognised as risk

factors (Cleland et al 1985a, Hodsman et al 1983, Packer et al 1986). Of patients identified at being at high risk in the SOLVD study on the basis of the presence of severe CHF, pre-treatment serum sodium of <130 mmol/L, or already prescribed a vasodilator, only 15% developed symptomatic hypotension (Hood et al 1991). In those with pre-treatment systolic blood pressure of less than 100mm Hg, symptomatic hypotension occurred in 42%, but syncope in only 17%.

These relatively easily identifiable, high risk individuals are now in the minority of those being commenced on ACE inhibitor therapy. Hyponatraemia and hypovolaemia are seldom found in those with mild to moderate CHF, for whom there are no reliable indicators of the likelihood of a significant fall in blood pressure. In a study of the acute effects of captopril in 36 patients with mild to moderate CHF, no pre-treatment clinical or laboratory parameter other than mean arterial blood pressure could be identified to predict the blood pressure response (Motwani et al 1994).

The possible role of the Bezhold-Jarisch reflex in the occurrence of first-dose hypotension has been suggested (Semple et al 1988). Activation of the reflex in man is associated with marked cardiovascular depression. As the authors point out, activation of the reflex can occur in response to a number of hormones and cardiovascular drugs. The risk of activation is increased by the upright posture and by high doses of diuretic. In addition it may be of relevance as far as ACE inhibitors are concerned that the reflex is activated by bradykinin (Kaufman et al 1980).

In spite of the increasing prevalence of CHF and the established role of ACE inhibitors in the treatment of the condition, direct comparisons of the haemodynamic response to various ACE inhibitor agents are rare. In addition, no large analysis of the pathophysiological determinants of the blood pressure response to initiation of therapy has

previously been carried out. This issue is addressed in the studies detailed in this thesis.

3.1.9.3 Renal effects of ACE inhibition in CHF

The RAS has an important role in regulating renal function in CHF. The effects of

activation of the RAS upon salt and water balance in CHF are central to the

pathophysiology of the condition. There is a strong correlation between angiotensin II and

aldosterone concentrations in CHF. Increased RAS activity and aldosterone levels result

in reduction of total body potassium and retention of sodium and water (Cleland et al

1987). The result is an increase in whole body sodium of 10-40% in untreated CHF.

However angiotensin II stimulated thirst and secretion of vasopressin (Goldsmith et al

1983) exacerbates water retention with total body water being 5-30% greater than normal.

The overall result is hyponatraemia. However hypokalaemia and hypomagnesemia also

result, changes which may be of relevance in the pathogenesis of arrhythmias.

The renal response to ACE inhibition is dependent upon a number of factors, in

particular the maintenance dose of diuretic, the degree of elevation of total body sodium

and water, the magnitude of the blood pressure fall and the severity of cardiac failure. In

patients with mild to moderate CHF, in whom pre-treatment GFR is only moderately

impaired, ACE inhibition commonly results in unchanged or even improved GFR. In

patients with more severe CHF, in whom GFR and effective renal plasma flow are

markedly impaired at baseline, in those in whom blood pressure is low, and in those on

high maintenance doses of diuretic, ACE inhibition often results in further deterioration in

GFR (Robertson & Richards 1987, Motwani et al 1994). A number of studies have

suggested that angiotensin II has a particularly important role in maintaining glomerular

filtration pressure when systemic blood pressure is low (Packer et al 1986a, Motwani et al

1994).

ACE inhibition corrects both hyponatraemia (Packer et al 1984) and total body potassium deficit (Cleland et al 1985b). Although angiotensin II helps to maintain glomerular filtration rate in CHF, there is a correlation between the degree of RAS activation on the one hand and hyponatraemia and blood urea concentration on the other (Brown et al 1970). Thus it appears that in CHF angiotensin II is essential to the maintenance of efferent arteriolar tone and thus glomerular filtration while at the same time responsible for the sodium retention of CHF. Motwani et al have suggested that there is, for each individual patient, an optimal level of inhibition of ACE which preserves sufficient efferent arteriolar tone to maintain glomerular filtration while reducing angiotensin II to a degree sufficient to allow natriuresis. In keeping with this suggestion is the observed attenuation of frusemide-induced natriuresis and reduction in GFR by high doses of captopril, while a low dose of captopril augment both GFR and frusemide induced natriuresis (Motwani et al 1992).

Differences exist in the renal responses to ACE inhibition and angiotensin II receptor blockade in rats after experimental MI (Deck et al 1996). While captopril and losartan elicited similar changes in arterial pressure, increases in renal blood flow and GFR, and decreases in urine flow rate and fractional excretion of sodium were less marked with losartan. Blockade of prostaglandin synthesis by indomethacin had no effect on renal haemodynamics in response to either ACE inhibition or angiotensin II receptor blockade. However, inhibition of bradykinin attenuated the effects of ACE inhibition on renal haemodynamics but was without effect on these parameters after angiotensin II receptor blockade. Blockade of bradykinin and of prostaglandin synthesis improved sodium excretion in both groups. These authors concluded that both the kinin and prostaglandin

systems are of importance in the regulation of renal function during suppression of the

RAS.

3.1.9.4 Cardiac effects

ACE inhibition elicits characteristic changes in cardiac function in CHF. As

peripheral vascular resistance falls, stroke volume increases with a net reduction in cardiac

work (Halperin et al 1982). Cardiac oxygen consumption is unchanged or falls. Heart rate

falls as a result of increased cardiac vagal (Osterziel et al 1990) and reduced cardiac

sympathetic (Mulligan et al 1989) activity. Myocardial oxygen consumption at rest is

reduced and lactic acid production reduced or unchanged (Chatterjee et al 1982). Any loss

of the inotropic effect of angiotensin II appears to be small and left ventricular function is

almost always improved after systemic administration of ACE inhibitor. Infusion of ACE

inhibitor into the coronary circulation of patients with CHF results in impairment of

contractility (Foult et al 1988). The beneficial effect of ACE inhibitors on sudden death in

CHF may be partly explained by a reduction in the frequency and severity of ventricular

arrhythmias (Webster et al 1985) as a result of reduced sympathetic nerve activity,

improved electrolyte balance or reduction of angiotensin II levels.

3.2 CHEMICAL STRUCTURE OF ACE INHIBITORS

The first orally active ACE inhibitor, captopril, was based on peptide elements

from the venom of the South American pit viper. Orally active inhibitors of the enzyme

were first synthesized in the late 1970's and were derived from the intravenous peptide

ACE inhibitors. At that time the perceived possible clinical use of these agents was in the

treatment of renovascular hypertension in which circulating levels of angiotensin II are

elevated. At the time of writing, the ACE inhibitors are extensively used in hypertension

but are for reasons outlined above relatively contraindicated in hypertension of renovascular origin. Latterly a central role in the treatment of CHF has been established for ACE inhibitors to the extent that these agents are now one of the mainstays of the treatment of the condition. The development of this class of drug has been based on the scientific evidence already discussed of the probable role of the renin-angiotensin system in these two common pathological conditions. A large number of ACE inhibitors have been synthesized over the last 25 or so years. At the present time in the UK., captopril, cilazapril, enalapril, fosinopril, lisinopril, perindopril, quinapril, ramipril and trandolapril are licensed for use.

The basic structure of the agents is based on a biochemical model of the active site of the enzyme. This is in turn based on the X-ray crystal structure of the enzyme carboxypeptidase A, closely related to angiotensin converting enzyme. A number of specific drug-enzyme interactions are thought to be important to binding affinity and specificity. These include occupation by part of the drug of a hydrophobic pocket formed by the tertiary structure of the enzyme; chelation of a zinc ion; and binding to a cationic resdue within the protein. Early ACE inhibitors possessed a sulphydryl group for the purpose of biding to zinc. This moiety has been associated with some of the unwanted side effects of ACE inhibitors, and second and third generation agents do not contain a sulphydryl group.

The affinity of ACE inhibitors for the enzyme is related to chemical class. The sulphydryl group of which captopril is an example binds strongly to the enzyme but rapid oxidation results in a short duration of action. Enalapril, cilazapril, lisinopril, quinapril, ramipril and perindopril bind to the enzyme via various carboxylic acid functions while fosinopril binds via its phosphinic acid group. The rigid N-terminal ring structure of the

first ACE inihibitor captopril, an important element determining binding affinity, has been retained although the proline structure of the early agents has been modified in a number of cases.

The majority of ACE inhibitors are synthesized as prodrug esters of the active diacid moiety, improving solubility and oral bioavailability. De-esterification of the prodrug occurs predominantly in the liver and in the gut wall. The duration of action of prodrug ACE inhibitors is delayed and prolonged compared to the diacid moiety. Of the two isomers of a given ACE inhibitor, only the trans isomer is pharmacologically active. Modified forms of agents incapable of undergoing isomerisation have been produced.

3.3 PHARMACOLOGY OF ACE INHIBITORS

3.3.1 Pharmacokinetics

3.3.1.1 General

Differences in pharmacokinetic properties among ACE inhibitor agents should logically influence their tissue distribution. As elements of the renin angiotensin system can be found in numerous tissues, the implications are that differences among ACE inhibitors in terms of their availability at tissue sites may result in differential effects.

The pharmacokinetics of ACE inhibitors are determined by a number of factors (Table 3.3). Most available ACE inhibitors are prodrug ester agents which undergo de-esterification to the active diacid metabolite. Administration as the ester increases oral bioavailability. Of the currently available agents, captopril and lisinopril do not require de-esterification. For the other agents, de-esterification takes place in the wall of the gastro-intestinal tract and liver. However relative lipophilicity of the parent ester may allow access to tissue sites from which the diacid metabolite is excluded.

The pharmacokinetic profile of ACE inhibitors is usually described by a distribution phase followed by an initial elimination phase of 2-8 hours and a prolonged terminal elimination phase often lasting over 24 hours. This latter elimination phase is thought to be due to dissociation of drug from binding sites, including tissue ACE. At high concentrations the amount of drug bound to ACE is relatively small and elimination is determined by drug clearance. At low drug concentrations, drug binding and dissociation is more relevent to the elimination rate. Drug half life is represented by separate values for the initial and terminal elimination phase. Due to saturation of binding sites ACE inhibitors do not accumulate with repeated dosing .

3.3.1.2 Tissue distribution

Binding of ACE inhibitor drug to the enzyme can be demonstrated in a variety of tissues. Differences in protein-binding characteristics should influence tissue distribution of ACE inhibitors. Johnston et al assayed displacement of the lisinopril derivative [125 I]351-A by different ACE inhibitors (Johnston et al 1989). The IC_{50} values of quinaprilat in plasma, lung, kidney and the heart were lower than those for benazeprilat, lisinopril, fosinoprilat or perindopril. The displacement of [125]351-A from membrane homogenates of rat heart by various ACE inhibitors revealed similar results (Fabris et al 1989). The overall rank order of potency was quinaprilat = benazeprilat > perindopril > lisinopril > fosinoprilat. The results of these two studies suggest that quinaprilat has a high affinity for cardiac ACE in comparison to other agents. The clinical relevance of such differences is unclear.

3.3.1.3 Metabolism

Table 3.3 Factors influencing pharmacokinetics of ACE inhibitors

Absorption/bioavailability
Transformation of prodrug
Lipophilicity

ACE inhibitor/ ACE kinetics

protein binding

Availability at tissue sites

Metabolism ➜ Active metabolites
         ➜ Inactive metabolites

Elimination ➜ renal
        ➜ hepatic

Table 3.4 Haemodynamic effects of ACE inhibitors

Arterial blood pressure ↓
Total peripheral resistance ↓
Cardiac output ↑/=
Heart rate =/↓

Renal blood flow ↑
Cerebral/coronary blood flow ↑

Cardiac/vascular hypertrophy ↓

Table 3.5 Incidence of symptomatic first-dose hypotension in trials of ACE inhibitor therapy in patients with congestive heart failure.

Investigator	Initial ACE inhibitor dose	No. of patients	Study design	Incidence (%)
Captopril Multicenter Research Group 1983	Captopril 25mg	96	mc, r, db, pc	33.3 [a]
Cleland et al 1985b	Enalapril 5mg, 10mg	26	nc	11.5
DiCarlo et al 1983	Enalapril 2.5mg	15	nc	13.3
Hasford et al 1991	Enalapril 2.5mg Prazosin 0.5mg	599 583	mc, r, nb, pc	5.2 12.9 (p<0.000012)
Packer et al 1986	Enalapril 10mg Captopril 25mg	42 42	r, nb, pc	4.8 [b] 0
SOLVD Investigators 1991	Enalapril 2.5mg	7402	mc, r, db, pc	2.2 [c]

[a] Incidence during a 2 day, single-blind captopril dose titration period prior to randomisation.

[b] Incidence after test dose of either ACE inhibitor.

[c] Incidence during 2-7 day single-blind enalapril titration prior to randomisation.

db=double-blind; mc=multicentre; pc=placebo controlled; nb=non-blind; r=randomised; nc=non-comparative. SOLVD=Studies of Left Ventricular dysfunction.

Table 3.6 Incidence of symptomatic first-dose hypotension in major clinical trials of ACE inhibitor therapy in patients after acute myocardial infarction.

Investigator	Initial ACE inhibitor dose	No. of patients	Study design	Incidence (%)
CCS-1 CG 1995	Captopril 6.25mg, 12.5mg	6814	mc, r, nb, pc	9.4 [a]
Swedberg et al 1992	Enalapril 2.5mg	3044	mc, r, db, pc	7
	Placebo	3046		2
ISIS-4	Captopril 6.25mg	29028	mc, r, pc	5.3 [b]
	Placebo	29022		2.5
Pfeffer et al 1992	Captopril 6.25mg	2250	mc, r, db, pc	0.7 [c]
Sharpe et al 1991	Captopril 12.5mg	114	r, db, pc	10.5 [c]

[a] Persistent hypotension at day 0-1.

[b] Hypotension necessitating termination of treatment at day 0-1.

[c] Incidence after test dose of captopril prior to randomisation.

CCS-1 CG=Chinese Cardiac Study Collaborative Group; ISIS-4=Fourth International Study of Infarct Survival. db=double-blind; mc=multicentre; pc=placebo controlled; nb=non-blind; r=randomised; nc=non-comparative.

Of the available ACE inhibitors lisinopril is excreted intact, the others being variably metabolised to inactive compounds. All of the available agents are eliminated via the kidney and will accumulate in renal impairment. Although a number of ACE inhibitors undergo hepatic metabolism, this is never sufficient to prevent any particular agent being accumulated in renal failure. Tailoring of the dose of ACE inhibitor to the GFR is therefore recommended. The more lipophilic agents (cilazapril, enalapril, quinapril, ramipril) undergo relatively greater hepatic metabolism. Captopril is metabolised to disulphide moieties which may regenerate captopril and prolong its duration of action.

3.3.1.4. ACE inhibitor/ACE interaction

There are two known binding sites for an ACE inhibitor drug on the enzyme (Perich et al 1991). Enzyme inhibition is competitive and complete inhibition of circulating ACE occurs at low circulating concentrations of ACE inhibitor. Following inhibition of ACE, circulating levels of angiotensin II fall, while angiotensin I and plasma renin concentrations rise.

During chronic ACE inhibition, some increase in plasma ACE levels occurs via enzyme induction. Sensitive assays of angiotensin peptide levels during chronic ACE inhibition have shown that the initial very low levels of angiotensin II are not maintained. This does not appear to be reflected in ACE inhibition as estimated using the traditional in vitro assay of plasma ACE activity. Thus traditional methods may overestimate the true extent of ACE inhibition. While this phenomenon was previously thought not to be relevant to the clinical use of ACE inhibitors, it has been suggested recently that escape of only a small percentage of ACE from inhibition may allow production of a patho-physiologically significant amount of angiotensin II, given the abundance of the enzyme

(Nussberger et al 1994).

3.3.2 Pharmacodynamics

Although it is generally accepted that the pharmacological effects of inhibition of ACE are mediated via a reduction in the amount of circulating angiotensin II produced from angiotensin I, the enzyme has as potential substrates a number of other peptides of which bradykinin may be the most clinically relevant. The haemodynamic effects of ACE inhibitors in patients with CHF have been described in section 3.1.7 and are summarised in Table 3.4. Broadly similar effects are seen in hypertension and to a lesser extent in normal man.

While it is accepted that the acute hypotensive response to ACE inhibition is due to reduction in circulating angiotensin II levels, the long-term blood pressure response does not correlate well with this parameter. There are a number of alternative modes of action of ACE inhibitors by which they may influence blood pressure and inhibition of ACE located at a site or sites other than in plasma is likely to be of relevance. As stated above, interaction between the RAS and sympathetic nervous systems appears to occur at a number of sites and may be of relevance to the haemodynamic effect of ACE inhibition. With chronic treatment, the negative sodium balance which results from suppression of aldosterone production may contribute.

Potentiation of the vasodilator effects of kinins and resultant effects on prostaglandins may be of relevance to the haemodynamic response to ACE inhibition (Carbonell et al 1988). The recent development of specific bradykinin B_2 receptor antagonists has allowed detailed exploration of the contribution of bradykinin potentiation to the effects of ACE inhibition. Data from animal studies support a role for bradykinin

potentiation in this regard. In the aortic banded hypertensive rat, the prevention of left

ventricular hypertrophy obtained with ramipril is prevented by the specific bradykinin B_2

receptor antagonist Hoe 140 (icatibant) (Linz & Scholkens 1992). Further to this, Hoe 140

reduces the antihypertensive effects of perindoprilat in spontaneously hypertensive rats,

the effect being more pronounced when the animals are exposed to a high salt diet

(Bouaziz et al 1994). To date no information is available on the relevance of these findings

to man.

3.3.3 Unwanted effects of ACE inhibition

The two best recognised unwanted effects of ACE inhibitor therapy are

hypotension, usually cited as occurring after the first dose, and renal impairment, the onset

of which appears to be rather more variable. The phenomenon of first-dose hypotension

has been discussed in section 3.1.9.2. The reported incidence of the phenomenon in the

major trials of ACE inhibition in CHF and after acute MI are shown in Table 3.5 and 3.6

respectively.

3.3.3.1 Renal impairment

As discussed above, the potential role of ACE inhibitors in the treatment of

diabetic nephropathy is currently of great interest. On the other hand, renal impairment is

widely perceived as a potential adverse event associated with ACE inhibitor therapy. The

risk in patients with uncomplicated hypertension appears to be very low, with no deaths

from renal failure being reported in over 11,000 such patients taking enalapril (Cooper et

al 1987). The overall incidence of ACE inhibitor induced renal impairment has been

reported as less than 1% (Inman et al 1988). A number of potential contributory factors

were identified in this study: hypovolaemia as a consequence of high doses of loop

diuretics, diarrhoea or vomiting; hyperkalaemia as a consequence of concomitant administration of potassium sparing agents or potassium supplementation; and concomitant administration of non-steroidal agents.

Proteinuria has been reported as a result of ACE inhibition, largely associated with the use of high doses of captopril in the early days of ACE inhibitor therapy (Frohlich et al 1984). Reports of proteinuria attributable to ACE inhibitor therapy are now rare. It appears likely that at least so renal damage previously attributed to ACE inhibitorme of the therapy was in fact due to the disease state for which the drug was prescribed (Captopril Collaborative Study Group 1982). The incidence of renal impairment secondary to the use of ACE inhibitors in CHF is unknown. As with the use of ACE inhibitors in hypertension, a number of confounding factors are relevant, not least of which the association between progression of chronic CHF and resultant deterioration in renal function.

3.3.3.2 Cough

The incidence of cough induced by ACE inhibitor therapy has been variously reported at between 5% and 25% of patients (Yeo & Ramsay 1990). As with hypotension, rather fewer patients withdraw from treatment as a result (Yeo et al 1991). The cough is typically dry, unproductive and worse at night. In controlled trials some improvement is obtained with non-steroidal compounds (Gilchrist et al 1989), suggesting potentiation of kinins and/or prostaglandins to be responsible. Cough as a side effect of ACE inhibition is more common among women and may be dose related. There is little evidence to suggest that the incidence of cough varies among ACE inhibitors.

3.3.3.3 Angioneurotic oedema

Angioneurotic oedema represents a rare unwanted effect of ACE inhibition. The

mechanism is unknown; patients present with swelling of the face and tongue or occasionally of the perineum and peripheries. The majority of episodes occur within 3 months of commencing treatment Hedner et al 1992).

3.3.3.4 Neutropenia

Neutropenia was initially described with high dose captopril therapy but has been reported with non-sulfhydryl containing agents. The incidence is related to the presence of renal impairment and more closely to the presence of collagen vascular disease (Cooper 1983). The majority of cases of neutropenia occur within 2-3 months of starting therapy. The effect is reversible with discontinuation of therapy.

3.3.4 Potential interactions of ACE inhibitors

3.3.4.1 Interaction between the RAS and the sympathetic nervous system.

There is a considerable body of evidence indicating a functional interaction between the RAS and the autonomic nervous sustem (ANS) (for review see Squire & Reid 1993). The earliest evidence of an interaction was the demonstration that angiotensin II can increase the release of catecholamines from the adrenal medulla (Braun-Menendez et al 1946). In the 1960s, facilitation of central (Bickerton & Buckley 1961) and peripheral (Zimmerman 1962) adrenergic neurotransmission was shown. Since then, interaction of the RAS and ANS at a number of sites has been demonstrated in a variety of species. Angiotensin II stimulates the superior cervical ganglion of a number of experimental animals and enhances noradrenergic transmission in a variety of isolated tissue and whole-animal preparations. A number of mechanisms have been proposed by which the interaction occurs, including presynaptic stimulation of noradrenaline release, increased

noradrenaline synthesis, reduced uptake, enhanced target cell responsiveness, and enhancement of transmitter release via stimulation of prejunctional angiotensin II receptors.

A striking feature of the use of ACE inhibitors in clinical practice is the lack of reflex tachycardia seen with these agents, in distinction to the effect of other vasodilators. This effect has been described as "parasympathomimetic" and has been demonstrated in hypertensive man (West et al 1989) and in CHF (Binkley et al 1993). Further to this, ACE inhibition improves the disturbed baroreceptor-cardiac reflex sensitivity seen after acute myocardial infarction (Marakas et al 1995), with relatively greater augmentation of parasympathetic activity than suppression of sympathetic activity. Thus evidence is accumulating that the interaction between the RAS and the ANS may be of patho-physiological significance. The site or sites of interaction remains unknown.

3.3.4.2 Effects mediated via bradykinin/prostaglandins

The possible role of bradykinin in mediating the effects of ACE inhibition continues to be the subject of much debate. It has been suggested that both the hypotensive effect of ACE inhibitors and the well recognised side effect of cough may be mediated via potentiation of bradykinin. As ACE catalyses the breakdown of bradykinin, ACE inhibition potentiates this hormone, which in turn leads to increased production of nitric oxide (NO), via stimulation of B_2-kinin receptors, and arachidonic acid metabolites such as prostacyclin and PGE_2. All of these act as vasodilators although in man the principle mediator appears to be NO (Bonner et al 1990). Plasma levels of bradykinin are not increased to any significant degree during ACE inhibition (Mombouli et al 1991). It is likely that the contribution of bradykinin to vascular tone is determined by local concentration of the

compound and the endothelial relaxing factors which it releases. In vitro studies show that vascular smooth muscle relaxation mediated via NO is augmented by ACE inhibition (Mombouli et al 1991).

Human studies regarding the relative contribution of bradykinin to the effects of ACE inhibition have been limited by the lack of orally active bradykinin antagonists. Studies in animals suggest that potentiation of bradykinin is of relevance to at least some of the effects of ACE inhibition. In spontaneously hypertensive rats, blockade of bradykinin receptors with Hoe 140 prior to administration of perindoprilat completely abolishes the hypotensive effect of the ACE inhibitor (Bouaziz et al 1994). Similarly, Hoe 140 abolishes the antihypertrophic effect of ramipril in aortic banded rats (Linz & Scholkens 1992).

3.3.4.3 Effects mediated via ANF

Atrial natriuretic factor (ANF) is a potent vasodilator substance which interacts with the RAS at a number of sites. In general terms ANF antagonises the secretion of renin and aldosterone, inhibition of angiotensin II-mediated sodium reabsorption from the proximal tubule, and attenuation of angiotensin II mediated vasoconstriction. Atrial natriuretic factor and the RAS show reciprocal responses to manipulations of sodium status (Tuchelt et al 1990).

Angiotensin converting enzyme may be involved in the degradation of ANF. In health, plasma levels of renin and aldosterone levels are inversely related to those of ANF. However in CHF both plasma renin levels and concentrations of ANF rise progressively as the condition progresses (Richards et al 1986). Infusion of ANF in animal models of heart failure suggest that the peptide can reverse the sodium retention and overall RAS

activation characteristic of the condition (Lee et al 1989). The antagonistic effect is lost in

severe heart failure (Riegger et al 1988). Inhibition of ACE, if sufficient to lower renal

perfusion pressure, reduces ANF-induced natriuresis (Gaillard et al 1989). The clinical

importance of these findings are doubtful in view of the findings of studies from infusion of

ANF in patients with CHF, showing little or no change in RAS parameters (Munzell et al

1991).

CHAPTER 4

GENERAL METHODS

4.1 LABORATORY METHODS

4.1.1. Drug concentrations

Concentrations of both ester and diacid ACE inhibitor were analysed using a standardised inhibition assay as described by Tocco et al (1982) and modified by Francis (Francis et al 1987). Endogenous ACE activity is inactivated by heating. A constant amount of exogenous angiotensin converting enzyme is incubated with the exogenous substrate Hip-His-Leu and the hippurate generated detected by high performance liquid chromatography (HPLC) to quantify the amount of inhibitor present. Rabbit plasma is used as the source of high activity ACE. Drug concentrations are determined against standard curves. Concentrations of ester prodrug ACE inhibitors are determined by alkaline degradation of an aliquot in addition to the determination of diacid ACE inhibitor. Subtraction of diacid from total substrate inhibition allows quantification of ester and diacid concentrations in a sample of plasma where both are present.

Solutions

Drug standards

Three 10 mg lots of drug were each dissolved in 100 ml distilled water to give solutions of 100 μg/ml. These solutions were then designated to the standard curve, and quality control 1 (QC 1) and quality control 2 (QC 2) respectively. Each was then diluted with distilled water to:

Standard curve 500 ng/ml

QC 1 100 ng/ml

QC 2 50 ng/ml.

20 µl of each of these working standards were each added to 480 µl blank plasma

(patient's own) giving solutions of the following concentrations:

Standard curve 20 ng/ml

QC 1 4 ng/ml

QC 2 2 ng/ml

For the construction of the standard curve, 20 ng/ml standard solution was diluted

down to 0.3 ng/ml, all dilutions being prepared in duplicate. An aqueous blank, control blank

(0.9% Na Cl) and plasma blank (100 µl plasma as used for standard curve) were then run in

addition to standard solutions as prepared above. Standard curves were fitted using non-linear

regression, 2 exponentials, to the equation:

$$Y = A. \exp(alpha.X) + B. \exp(beta.X)$$

Sample peak areas were read from the fitted line using an in-house programme,

Newton 2.

5 mM Hip-His-Leu (Exogenous substrate)

21.48 mg Hip-His-Leu was dissolved in 10 ml assay buffer. The substrate shows

gradual degradation to produce hippuric acid and the substrate solution was freshly made

for each assay.

100 mM potassium phosphate buffer pH 8.3

4.375g of NaCl and 5.705g of K_2HPO_4 were dissolved in 250ml distilled water. 1.361

of KH_2PO_4 was dissolved in 100ml distilled water. The monopotassium salt was added to the dipotassium salt until the pH reached 8.3.

Hippuric acid standards

179.2 mg of hippuric acid was added to 100ml distilled water. Two ml of this solution was then taken and added to 18 ml of drug-free plasma. A standard calibration curve over the concentration range 0.05 to 1.0 mmol/L hippurate was prepared.

Internal standard

20.4 mg phthalic acid was dissolved in 20 ml methanol and 80 ml of distilled water added. The solution was then diluted 1:2.5 with distilled water to give the standard solution of 0.41 mmol/L.

Mobile phase

5.44 g KH_2PO_4 was dissolved in 1.5 litres of distilled water and the pH adjusted to 4.0 with orthophosphoric acid. The solution was made up to a total of 2 litres with distilled water and 140 ml of the solution replaced with 140 ml of methanol. The mobile phase was filtered through a 0.8 micron aqueous filter and degassed by bubbling through with helium.

Analytical procedures

Procedure for diacid metabolites

1. A sample of standard or patient plasma, 100 µl in duplicate, was inactivated by heating at 60°C for 1 hour.

2. Add 400 µl of substrate in buffer.

117

3. Add 50 µl diluted rabbit serum.

4. Incubate at 37°C for 45 minutes.

5. Add 100 50% (v/v) HCl. Vortex briefly.

6. Add 100 µl (IS).

7. Add 50 mg NaCl. Vortex briefly.

8. Add 1 ml ethyl acetate. Vortex briefly.

9. Centrifuge at 2000 rpm for 5 minutes.

10. Remove 500 µl of the organic layer into a clean tube.

11. Concentrate under air at 37°C. Redissolve in 150 µl of mobile phase.

12. Inject 50 µl aliquot.

Procedure for total drug concentration

This measures total drug concentration (i.e. parent ester + diacid metabolite).

Concentration of parent ester is obtained by subtraction of diacid concentration from total

drug concentration.

1. Take 100 µl samples (prepared as above) of standard or patient plasma in duplicate.

2. Add 100 µl rat serum.

3. Incubate at 37°C for 1 hour

4. Inactivate by heating at 60°C for 1 hour.

5. Continue as for steps 2-12 above.

HPLC conditions

Column: Spherisorb 5µm ODS 1 cartridge 10 cm x 4mm

Mobile Phase: 20 mmol KH$_2$PO$_4$/L, pH 4.3, in methanol 93:7

Flow: 2.5 ml/min giving approximate column pressure of 1000 p.s.i.

Detector: Ultraviolet, 228 nm, Range 0.16 AUFS.

Injection volume: 50 µl.

Approximate retention times:

IS   1.5 minutes

Hippuric Acid 2.5 minutes

The assay is not drug specific and was applied to the quantification of perindopril, perindoprilat, enalapril, enalaprilat, quinapril and quinaprilat in the studies reported here. Intra- and inter-assay variability was approximately 3-5 and 5-8% respectively for both metabolite and total drug. The lower limit of drug detection was 0.1ng/ml for both metabolite and total drug.

4.1.2. Angiotensin converting enzyme activity

Angiotensin converting enzyme activity (ACE) was determined by the method of Chiknas (1979). Plasma ACE activity is estimated using the measuremnt of hippuric acid generated from the exogenous synthetic substrate Hippuryl-Histidyl-Leucine (Hip-His-Leu). The hippuric acid produced by the action of ACE on this substrate is quantified against an internal standard by HPLC. This gives an indirect index of ACE activity. One unit of ACE activity (1 EU/L) is that amount of ACE producing 1 mole of hippuric acid per minute at 37°C under controlled reaction conditions in vitro.

Analytical procedure

Procedure for standards

Into 4 ml polypropylene tubes were placed (in duplicate)

1. 200 μl substrate

2. 50 μl 50% HCL (v/v)

3. 20 μl standard plasma

4. 50 μl internal standard solution. Vortex briefly.

5. 50 mg NaCl. Vortex briefly.

6. 1 ml ethyl acetate. Vortex for 15 seconds.

7. Centrifuge at 2000 rpm for 5 minutes.

8. Remove 500 μl of the organic layer to a clean polypropylene tube and concentrate under air/N_2 at 37°C.

9. When dry add 100 ul of mobile phase and vortex briefly.

10. Inject 20 μl onto HPLC system.

Procedure for unknown samples

Into 4 ml polypropylene tubes were placed (in duplicate)

1. 200 μl 5 mmol Hip-His-Leu in substrate buffer.

2. 20 μl unknown plasma sample. Vortex briefly.

3. Incubate in water bath at 37°C for 30minutes.

4. Stop the reaction by adding 50 μl 50% HCl and vortexing.

5. Steps 4-10 as for procedure for internal standard solution.

The minimum limit of detection was 0.2 EU/L. The accuracy of the method as determined using a quality control sample provided by Sigma Chemicals was 104%. Analysis of 10 replicates of this 18 EU/L standard gave figures of 2.0 and 3.3% for intra- and inter-assay variability respectively.

4.1.3 Plasma renin activity

Plasma renin activity was determined by radioimmunoassay of angiotensin I formed

120

from exogenous angiotensinogen (Derkx et al 1979). The detection limit of this sensitive and specific assay (renin activity) was 0.1 ng AI/ml/hr. Inter assay coefficient of variation was 7%.

4.1.4 Plasma aldosterone

Plasma aldosterone was measured using an established radioimmunoassay based on a commercial kit (Aldosterone MAIA, Biodata, SPA, Italy). Intra-assay coefficient of variation was 2.5%, inter-assay coefficient of variation 6%.

4.2 CLINICAL STUDIES

4.2.1 Blood pressure and heart rate measurement

In the clinical studies described, procedures for the measurement of blood pressure and heart rate and for blood sampling were identical. Blood pressure and heart rate were recorded using a validated (Johnson and Kerr 1985) semi-automatic device (Sentron, Bard, Sunderland, U.K.). This device was calibrated and maintained by the Clinical Physics Department of the Western Infirmary. The device utilises an oscillometric technique for indirect measurement of blood pressure.

All subjects had baseline blood pressure recordings made after 30-45 minutes supine rest. Recordings were made at 2 minute intervals to establish baseline values and in triplicate at pre-determined time points (5, 10, 15, 20, 30, 40, 50 minutes and at 1, 2, 3, 4, 5, 6, 8, 10 and 24 hours) after administration of a single dose of study medication during each study for comparison of drug effects.

4.2.2 Blood sampling

121

Blood sampling for the assessment of neurohumoral parameters was carried out at pre-determined time points in each study (5, 10, 15, 20, 30, 40, 50 minutes and at 1, 2, 3, 4, 5, 6, 8, 10 and 24 hours after administration of a single dose of study medication). Blood was sampled into chilled tubes as appropriate and immediately centrifuged at -4° C for 15 minutes at 3000 rpm. Plasma was then separated and stored at -70° until assay.

4.2.3 Study protocols

All studies described here were approved by the local regional Research and Ethical Committee after submission of detailed protocols. All studies were reviewed and granted approval by this committee prior to their commencement. All subjects were screened for suitability for inclusion in the relevant study. Suitability in terms of the meeting of specific inclusion and exclusion criteria, physical examination and clinical history was determined by myself. Each subject was given a full verbal explanation of the purpose of the study and the procedure involved and was also provided with a written information sheet detailing the aims and procedures of the study prior to written informed consent being obtained.

4.3 Statistical Analysis

Blood pressure and heart rate were measured in triplicate at pre-determined time points in each study. Mean arterial pressure was calculated using the standard formula

$$MAP = DBP + (SBP+DBP)/3.$$

The mean of the three recordings of systolic, diastolic and mean blood pressure was taken as the value used for statistical analysis. All blood pressure data are expressed as mm Hg and all heart rate data as beats per minute. Heart rate, mean arterial pressure, systolic blood pressure

and diastolic blood pressure were compared both as absolute values and after correction for baseline values. The values obtained were analysed using repeated measures analysis of variance using a statistical package (RUMMAGE) on an ICL 3988 mainframe computer. The model used contained two fixed factors (time with variable levels, and treatment with variable levels) and one random factor (patients, with 12 levels for each of the heart failure studies). Treatment-time interactions were estimated using Bonferroni correction for repeated comparisons, comparing only treatments at each set time point. No within treatment comparisons at different time points were carried out.

All heart rate and blood pressure data were subjected to baseline correction by subtraction of pre-treatment values from the value at each time point before statistical analysis. Statistical significance was assumed if $p < 0.05$ in all cases. Angiotensin converting enzyme activity was calculated from the formula:

% inhibition = 100 x (1-ACE activity/pre-treatment ACE activity).

Graphical representation in all cases represents the mean value for each treatment group at each time point. Error bars represent one standard deviation of the mean. For all graphs, time after dosing is shown on a linear scale on the x-axis. Twenty four and forty eight hour values are shown separately on the right of the x-axis where appropriate.

Baseline laboratory indices and subject demographic data were compared amongst treatment groups by Freidman one way analysis of variance. Comparison of the New York Heart Association classification of heart failure severity was achieved using Kruskal-Wallis analysis of variance by ranks.

COMPARISON OF THE HAEMODYNAMIC RESPONSE TO INITIATION OF ACE INHIBITION WITH ORAL PERINDOPRIL , ORAL ENALAPRIL OR INTRAVENOUS PERINDOPRILAT IN CONGESTIVE HEART FAILURE

5.1 INTRODUCTION

This section describes the comparative haemodynamic effects of initiation of ACE inhibitor therapy with a number of agents in patients with stable, chronic CHF. A randomised, double-blind, parallel group, placebo controlled comparison of the effects of the established ACE inhibitor enalapril with the new, long-acting agent perindopril and its active diacid metabolite perindoprilat was carried out in 48 patients with congestive heart failure.

Previous work from this Department has demonstrated qualitative and quantitative differences in the first dose haemodynamic response to ACE inhibitors in CHF. Whereas enalapril 2.5mg p.o. produced a significant fall in supine mean arterial pressure, the blood pressure profile seen following perindopril 2mg p.o. did not differ from that seen after placebo. These differences were seen despite similar profiles of plasma ACE inhibition (MacFadyen et al 1991b). In a separate study, no such differential blood pressure responses were seen to the intravenous administration of the diacid compounds enalaprilat and perindoprilat in patients with heart failure (MacFadyen et al 1993). With regard to the haemodynamic response to the initiation of ACE inhibition, the aims of the study were:

1. To confirm a difference in haemodynamic response between perindopril 2mg p.o. and enalapril 2.5mg p.o.in elderly patients with stable, chronic CHF.

2. To establish whether oral perindopril treatment is associated with a fall in blood pressure between 10 and 48 hours after initiation of treatment and to compare this

response with that after enalapril. A fall in blood pressure occuring after a dose other than the first dose of ACE inhibitor would have important implications for the clinical use of such a compound.

3. To establish the haemodynamic response to a low dose of perindoprilat, 0.167mg, administered by constant rate intravenous infusion over 1 hour.

Additional aims were:

4. To investigate the neurohormonal response to each therapy.

5. To establish the pharmacokinetic profiles of the three active therapies in this population.

A consistent finding in a previous study was an initial rise in blood presure over the first hour of a constant rate intravenous infusion of both enalaprilat and perindoprilat (MacFadyen et al 1993). It was postulated that displacement of angiotensin peptides from binding sites on ACE may be responsible for this phenomenon. Thus, the present study was designed to investigate further this phenomenon. In the previous study, infusion of diacid ACE inhibitor had to be terminated prematurely in a number of patients due to excessive blood pressure falls. In the present study the ACE inhibitor agent was infused at the same rate as previously but over 1 hour only, ie 1/6 of the maximum dose in the previous study. The aims were to avoid the need to interrupt infusion and thus ensure all patients received the same dose whilst not altering any parameters which might contribute to an early rise in blood pressure.

5.2 PATIENTS AND METHODS

An observational study was carried out in patients with chronic, stable congestive heart failure (n=48, 51-85 years; 34:14, M:F), New York Heart Association (NYHA)

functional class II-IV. All patients were symptomatic and receiving stable doses of diuretic therapy (>80 mg frusemide or equivalent daily) prior to the study. Patients were recruited from the medical wards and from the general medical and cardiology clinics of the Western Infirmary. Each patient was admitted to hospital for elective commencement of ACE inhibitor therapy. All had erect systolic blood pressure >100mm Hg and none had significant disturbance of renal (plasma creatinine > 250umol/L) or hepatic (γGT > 3x upper limit of normal, transaminase > 2x upper limit of normal) function. The study was approved by the local ethical committee and written informed consent to participate in the study was obtained in all cases. The diagnosis of CHF was confirmed by full clinical history and by physical examination in all cases. All subjects had echocardiography performed to confirm impaired left ventricular function. A number had also undergone cardiac catheterisation and/or radionuclide scanning. Patient characteristics, by treatment group, are shown in Tables 5.1-5.4.

The haemodynamic status of each patient was assessed using the scoring system shown in Table 5.5. This allowed an estimate of the comparative status of fluid balance to be made among groups. All diuretic therapy was withdrawn for 48 hours prior to, and for the duration of, the study. All concomitant vasodilator therapy (oral nitrate, calcium channel blocker) and β-receptor antagonist therapy was witheld on the two days of the study. Digoxin, where prescribed, was continued. On the first study day each patient rose, washed and had breakfast as normal. A heparinised peripheral venous cannula was inserted in an antecubital vein for the purpose of blood sampling and in a vein of the contralateral forearm for infusion of intravenous study treatment (perindoprilat 0.167mg or placebo). The patients then returned to bed where they rested supine. Blood pressure was recorded at 2 minute intervals using a semi-automatic sphygmomanometer (Sentron,

Table 5.1

Demographic details
Placebo

Study No.	Age	Sex	Aetiology of CHF	NYHA Class	Cardiac Rhythm
4	85	F	IHD	II	SR
5	72	F	IHD	III	SR
9	76	M	IHD	III	AF
16	80	F	IHD	III	_ AF
20	74	F	IHD	II	AF
21	76	M	IHD	III	AF
28	65	M	HT	II	SR
29	77	M	VALVE	II	SR
36	80	F	IHD	III	SR
39	76	M	IHD	II	AF
44	64	M	HT	II	AF
48	72	M	IHD	III	AF

NYHA = New York Heart Association
IHD = Ischaemic heart disease
HT = Hypertension
VALVE = Valvular heart disease
AC = Alcohol related cardiomyopathy

Patient 29: Valve lesion = severe mitral regurgitation

Table 5.2

Demographic details
Perindopril 2mg po

Study No.	Age	Sex	Aetiology of CHF	NYHA Class	Cardiac Rhythm
3	71	M	IHD	III	SR
8	66	M	IHD	III	AF
12	79	F	IHD/HT	II	AF
15	75	M	IHD	II	SR
17	78	M	IHD	III	AF
24	69	M	AC	III	AF
27	73	F	IHD	III	SR
30	52	M	IHD/AC	III	SR
34	69	M	IHD	III	SR
40	71	F	IHD	II	SR
41	64	M	IHD	II	SR
45	81	F	IHD	III	SR

NYHA = New York Heart Association
IHD = Ischaemic heart disease
HT = Hypertension
VALVE = Valvular heart disease
AC = Alcohol related cardiomyopathy

Table 5.3

Demographic details
Enalapril 2.5mg po

Study No.	Age	Sex	Aetiology of CHF	NYHA Class	Cardiac Rhythm
2	75	F	IHD/VALVE	III	AF
7	75	M	IHD	II	SR
11	70	M	IHD/VALVE	II	- AF
13	55	M	IHD	III	SR
18	71	M	AC	IV	AF
22	76	M	IHD	II	SR
26	66	M	IHD	IV	SR
32	72	M	IHD	II	SR
35	66	M	IHD	II	SR
38	69	F	IHD	II	SR
43	75	M	IHD	III	SR
47	77	M	IHD	II	AF

NYHA = New York Heart Association
IHD = Ischaemic heart disease
HT = Hypertension
VALVE = Valvular heart disease
AC = Alcohol related cardiomyopathy

Patient 2: Valve lesion = severe mitral and tricuspid regurgitation
Patient 11: Valve lesion = severe mitral regurgitation

Table 5.4

Demographic details
Perindoprilat 0.167mg iv

Study No.	Age	Sex	Aetiology of CHF	NYHA Class	Cardiac Rhythm
1	67	F	VALVE/HT	II	AF
6	54	F	IHD	II	SR
10	62	M	IHD	III	SR
14	61	F	IHD	III	SR
19	76	M	IHD	III	AF
23	53	M	AC	III	AF
25	77	M	IHD	III	AF
31	69	M	IHD	II	SR
33	71	M	IHD	III	AF
37	69	M	IHD	III	SR
42	71	M	IHD	III	SR
46	60	M	IHD	II	SR

NYHA = New York Heart Association
IHD = Ischaemic heart disease
HT = Hypertension
VALVE = Valvular heart disease
AC = Alcohol related cardiomyopathy

Patient 1: Valve lesion = mitral + aortic regurgitation

Bard, Sunderland, UK) to establish baseline values.

After at least 45 minutes supine rest, blood was drawn for estimation of baseline

drug concentration, plasma ACE activity, plasma renin activity, aldosterone and

angiotensin peptide levels. Study medication was then administered double-blind:

intravenous therapy was administered as a constant rate intravenous infusion over 1 hour

and oral therapy, in the form of the standard formulation of each drug within an opaque

gelatin capsule, was administered at the same time as the intravenous infusion was

commenced. Study drugs were administered in accordance with the randomisation

schedule held by the department of pharmacy. Each patient received one of:

Perindopril 2mg p.o. + placebo i.v.

Enalapril 2.5mg p.o. + placebo i.v.

Placebo p.o. + perindoprilat 0.167mg i.v.

Placebo p.o. + placebo i.v.

After administration of study drug, supine blood pressure was measured at 2

minute intervals with additional triplicate determinations at pre-determined time-points.

Blood samples were withdrawn at frequent intervals for determination of pharmacokinetic

profile, angiotensin I and II levels, plasma renin activity and plasma ACE activity. Patients

remained supine until 10 hours after dosing during which time they received meals as

normal. At the end of this period they were allowed to rise and resume normal activity.

Prior to further blood sampling and blood pressure recording 24 hours after dosing,

the patients returned to bed where they rested supine for at least 45 minutes. At this point

a second oral dose of the study drug was administered. This was identical to that given on

day 1 in all cases except those patients who had received perindoprilat i.v. on day 1 (and

in whom oral therapy was placebo) who instead received perindopril 2mg p.o. on day 2.

Patients were fitted with a pre-programmed ambulatory blood pressure device (Spacelabs

90207) and allowed to resume normal activities.

Twenty four hours after administration of dose 2, the ABP device was removed and after a further period of supine rest blood was drawn for determination of ACE activity, drug levels, plasma renin activity and angiotensin peptides. At this point the randomisation code was broken by the department of pharmacy to reveal only if the patient had received active or placebo therapy. Those patients who had received active therapy were continued on open ACE inhibitor therapy (the majority receiving enalapril 5mg b.d.). Patients who had received placebo were given a standard test-dose of captopril 6.25mg p.o., followed by open ACE inhibitor therapy thereafter. No patients were included in any other study. Blood sampling for routine haematology and biochemistry was carried out prior to commencement of the study and at the end of the 48 hour study period.

5.3 RESULTS

5.3.1 General

The study groups were well matched in terms of haemodynamic status (Table 5.5) baseline MAP, pre-treatment diuretic dosage, biochemical and haematological indices and NYHA class (Table 5.6). The patients in the placebo group were older (Mean age 74.6±6.1 years) compared to those in the active treatment groups (oral perindopril 69.1±9.1; i.v. perindoprilat 65.8±8.0; oral enalapril 70.1±6.1 years). The ages of patients among the active treatment groups were similar (p=0.204).

No significant changes occurred in any biochemical indices over the study period (Table 5.6). As in previous studies employing similar protocols in the Department, a modest fall in haemoglobin concentration of approximately 1g/dl was seen and is attributable to blood sampling. None of the patients reported any symptoms during

Table 5.5

	Placebo	Perindopril	Enalapril	Perindoprilat	p value (ANOVA)
SCORE	6.8±5.4	5.2±2.1	5.7±3.4	6.2±2.6	0.738

Scoring system for assessment of haemodynamic status

SCORE:	0	1	2	3
Heart rate	<100	100-115	116-130	>130
Lung crepitations	None	Basal	Mid zones	Above MZ
JVP(cm above sternal angle)	0	0-2	>2 - angle jaw	> angle jaw
Oedema	None	Trace	Pretibial	Above knes/Ascites
Hepatomegaly	None	< 2 fb	> 2 fb	> 2 fb

Table 5.6
Summary statistics: Baseline laboratory values and change over study period , diuretic dose, NYHA class.
Mean (1 SD)

	Placebo	Enalapril	Perindopril po	Perindoprilat iv	p value (ANOVA)
Age	74.6 (6.1)	70.1 (6.2)	69.1 (9.1)	65.8 (8.0)	0.046
Sodium (mmol/l)	136 (4.5)	137 (3.6)	139 (4.6)	138 (3.8)	0.513
δ sodium	0.6 (3.6)	-0.5 (4.2)	0.0 (3.1)	-2.4 (4.1)	0.250
Potassium (mmol/l)	4.4 (0.7)	4.2 (0.4)	4.2 (0.4)	4.1 (0.6)	0.520
δ potassium	-0.1 (0.4)	0.2 (0.5)	0.3 (0.4)	0.0 (0.4)	0.137
Urea (mmol/l)	11.1 (4.0)	8.9 (5.3)	9.0 (4.1)	8.7 (2.9)	0.455
δ urea	-2.1 (3.5)	-0.6 (2.6)	-0.9 (2.3)	-1.0 (0.9)	0.480
Creatinine (μmol/l)	125 (40)	123 (34)	113 (31)	122 (23)	0.875
δ creatinine	-12 (17)	-6 (13)	-1 (18)	-13 (20)	0.260
Hb (g/dL)	13.2 (1.9)	12.9 (1.4)	14.4 (1.0)	14.6 (1.3)	0.016
δ Hb	-0.9 (0.8)	-1.2 (1.0)	-1.2 (0.7)	-1.1 (0.9)	0.802
NYHA (Average rank)	21.5	27.7	21.5	27.3 '	0.530 (Kruskall-Wallis)
Diuretic dose (mg frusemide /day)	97 (35)	106 (68)	85 (23)	90 (41)	0.696

supervised diuretic withdrawal or over the study period. Similarly the period of diuretic withdrawal was in no case associated with clinical evidence of decompensation of CHF. All left hospital after initiation of open ACE inhibitor therapy and reinstitution of diuretic and other medication.

5.3.2 Haemodynamic response

5.3.2.1 Baseline

Baseline mean blood pressures were similar in each treatment group. Mean arterial pressures for the 4 treatment groups were: placebo/placebo 89.5±11 mmHg; perindopril/ placebo 89.6±10 mm Hg; placebo/perindoprilat 90.7±12 mm Hg; enalapril/ placebo 87.9±13 mmHg (p=0.947). The baseline heart rates did not differ between groups: placebo/placebo 71 bpm; perindopril/placebo 76 bpm; placebo/perindoprilat 79 bpm; enalapril/ placebo 73 bpm (p=0.332).

5.3.2.2 1st Dose response
Blood pressure response

In three of the treatment groups, a transient rise in blood pressure was observed over the early part of the study period immediately following administration of study medication. The group receiving intravenous perindoprilat + oral placebo did not show this response. This phenomenon was evident for mean arterial pressure (Figure 5.1), for systolic pressure (Figure 5.2) and for diastolic pressure (Figure 5.3). This change did not reach statistical significance in any treatment group. Values shown are mean (1 SD)

A. Placebo p.o.+ Placebo i.v.

Following the administration of placebo therapy, and as in previous studies, a diurnal variation in mean arterial pressure was observed. Mean arterial pressure fell by 7.2
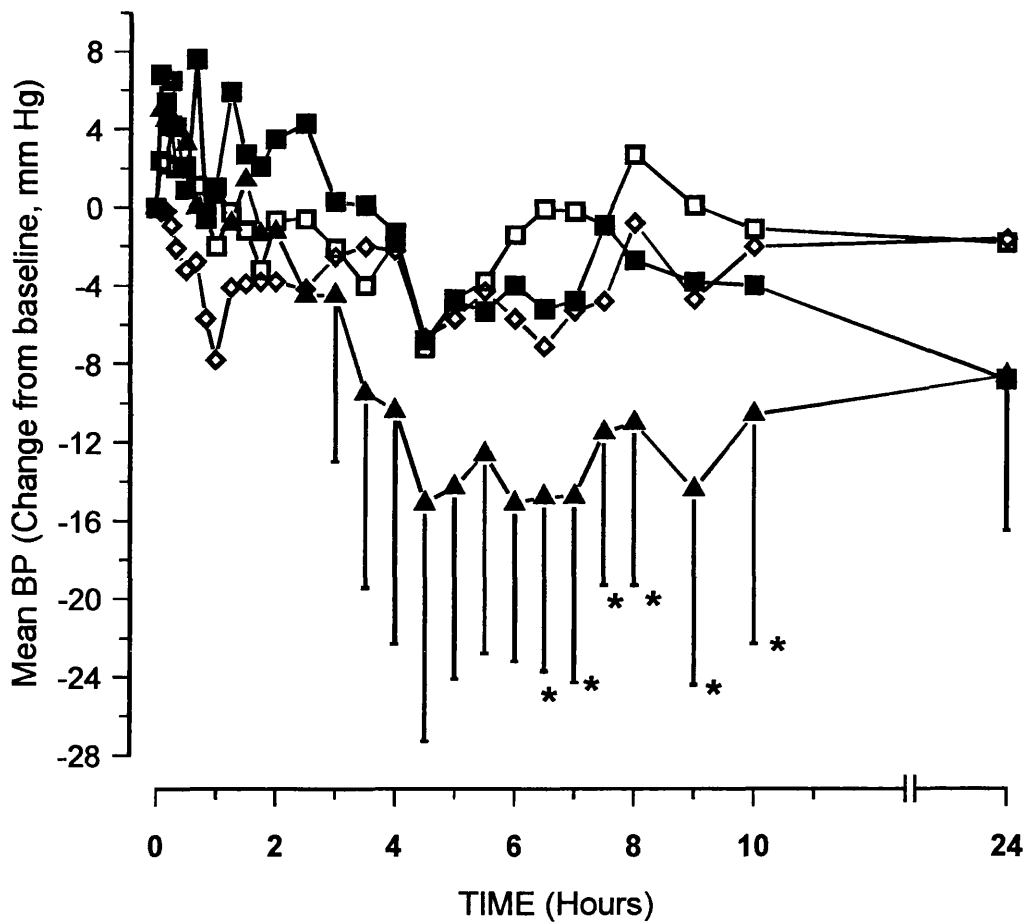
Figure 5.1:
Mean arterial pressure (Change from baseline, mm Hg) following a
single dose of placebo p.o. (□), enalapril 2.5mg p.o. (▲), perindopril
2mg p.o. (■) or perindoprilat 0.167mg i.v. (◇) in patients with congestive
heart failure.
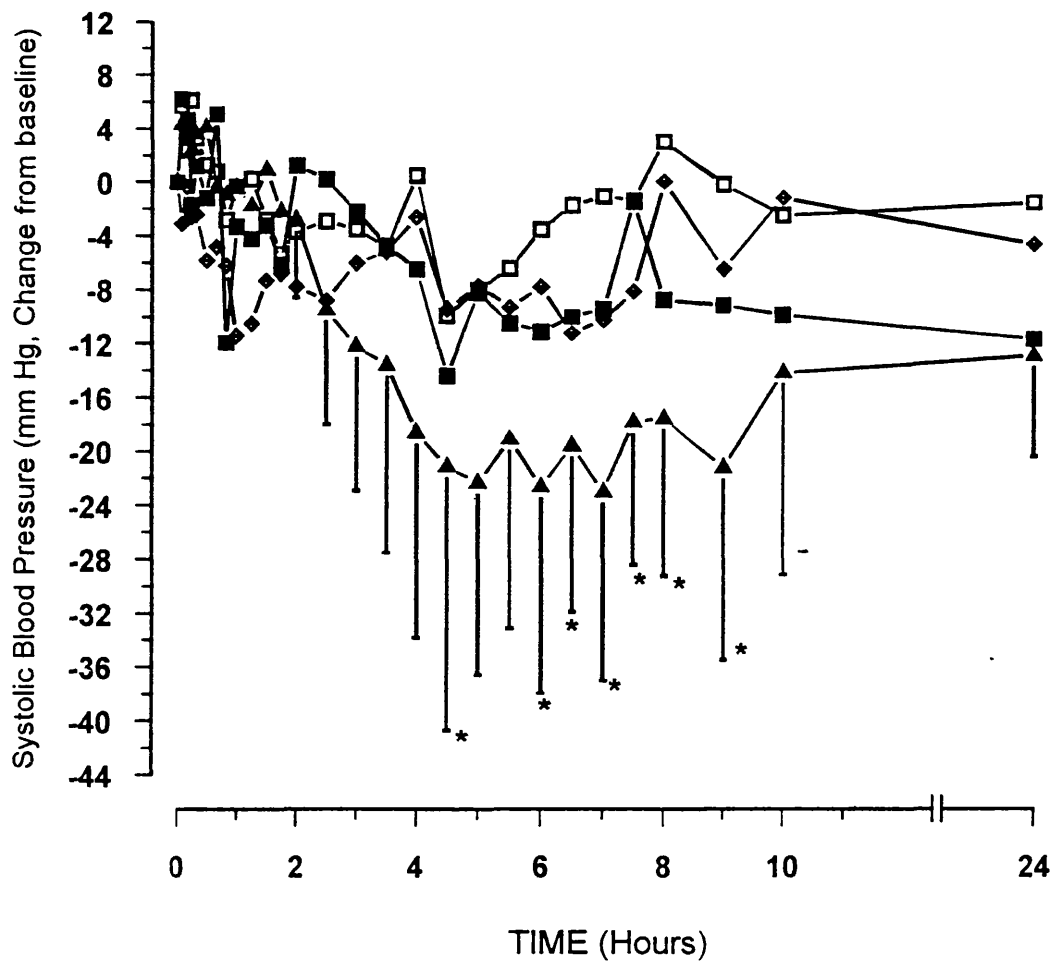
Figure 5.2:
Systolic blood pressure (Change from baseline, mm Hg) following placebo p.o. (□), enalapril 2.5mg p.o.(▲), perindopril 2mg p.o. (■) or perindoprilat 0.167mg i.v. (◇) in patients with congestive heart failure.
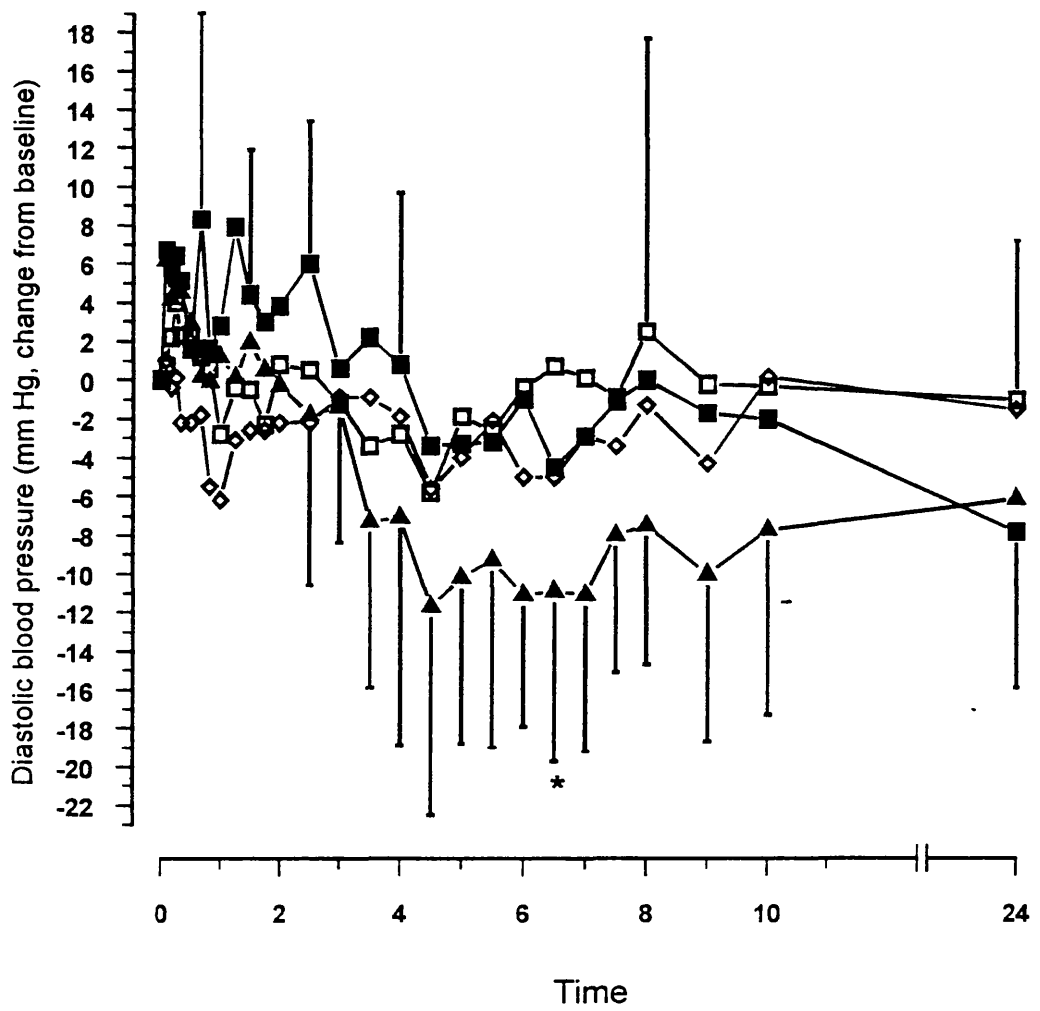
Figure 5.3:
Diastolic blood pressure (change from baseline, mm Hg) following placebo p.o. (□), enalapril 2.5mg p.o. (▲), perindopril 2mg p.o. (■) or perindoprilat 0.167mg i.v. (◇) in patients with congestive heart failure.
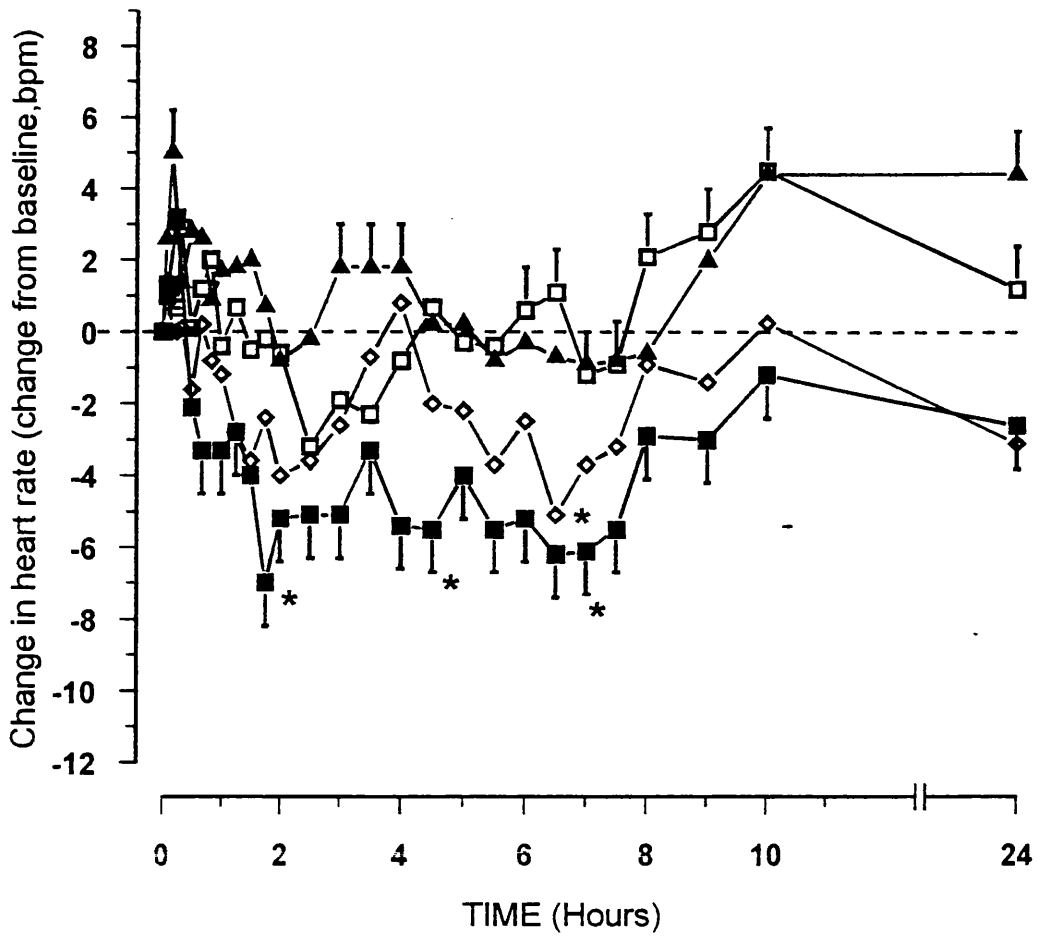
Figure 5.4:
Heart rate (Change from baseline, bpm) following a single dose of
placebo p.o. (□), enalapril 2.5mg p.o. (▲), perindopril 2mg p.o. (■),
or perindoprilat 0.167mg i.v. in patients with congestive heart failure.

(9.6) mm Hg over the first 4.5 hours and returned towards baseline thereafter. Mean arterial pressure at 24 hours was similar to baseline (Figure 5.1).

B. Enalapril p.o. + placebo i.v.

Enalapril 2.5mg p.o produced a sustained fall in mean arterial pressure which was significantly different from placebo between 6 and 9 hours post dose (Figure 5.1). The maximum mean fall in MAP was 15.1 (12.2) mm Hg, occurring at 6 hours post dose. A similar profile of blood pressure response was observed for systolic blood pressure. Systolic blood pressure fell significantly compared to placebo between 4 and 9 hours post-dose (Figure 5.2). The fall in diastolic blood pressure was lower in absolute terms than the systolic fall and was significantly different from placebo at 6.5 hours post dose (Figure 5.3). At 24 hours post-dose, MAP was approximately 9 mm Hg lower than at baseline. The difference was not significant.

C. Perindopril p.o. + placebo i.v.

In contrast to the marked blood pressure fall seen following enalapril, the MAP profile seen after perindopril 2mg p.o. did not differ from that seen with placebo (Figure 5.1). The maximum mean fall in MAP was 6.8 (7.9) mm Hg at 4.5 hours post dose. Similar patterns were observed for systolic and diastolic blood pressure. At 24 hours post-dose, MAP was approximately 9 mm Hg lower than baseline. The difference was not significant.

D. Placebo p.o. + perindoprilat i.v.

An early fall in MAP was observed over the 1 hour period of the infusion of perindoprilat 0.167mg. The fall did not reach statistical significance. At the end of the

infusion period an immediate though small "rebound" of MAP toward baseline was observed (Figure 5.1). Thereafter the BP profile was similar to that for placebo. The maximum mean BP fall was 7.8 mm Hg at 1 hour. Similar patterns were observed for systolic and diastolic blood pressure. Mean arterial pressure at 24 hours was similar to baseline.

Mean maximum BP fall

The mean maximum fall in MAP, irrespective of its time course was calculated for each treatment group. Values were: placebo 15 mm Hg (range 5-30); enalapril 21 mm Hg (range 4-30); perindopril 16 mm Hg (range 4-30); perindoprilat 14 mm Hg (range 6-25). There were no statistically significant differences among the treatment groups (ANOVA, p=0.126).

Heart rate response

Mean supine heart rate remained unchanged throughout the first 24 hours after the first dose of placebo and after the first dose of enalapril 2.5mg p.o. Heart rate fell following perindopril 2mg p.o, the change being significantly different from placebo at 2, 4.5 and 6.5 hours post dose. Heart rate fell following perindoprilat 0.167mg, the change being significantly different from placebo at 5.5 hours post dose (Figure 5.4).

5.3.2.3 Response to the 2nd dose

Blood pressure response

There were no significant differences in MAP among groups either at baseline or at 24 hours after dose 1. The change in blood pressure over day 2 of the study was compared to baseline (i.e. blood pressure prior to administration of the first dose of study medication). The fall in MAP over day 2 of the study was not significant, compared to placebo, for any treatment group (Figure 5.5). Similar results were obtained for systolic
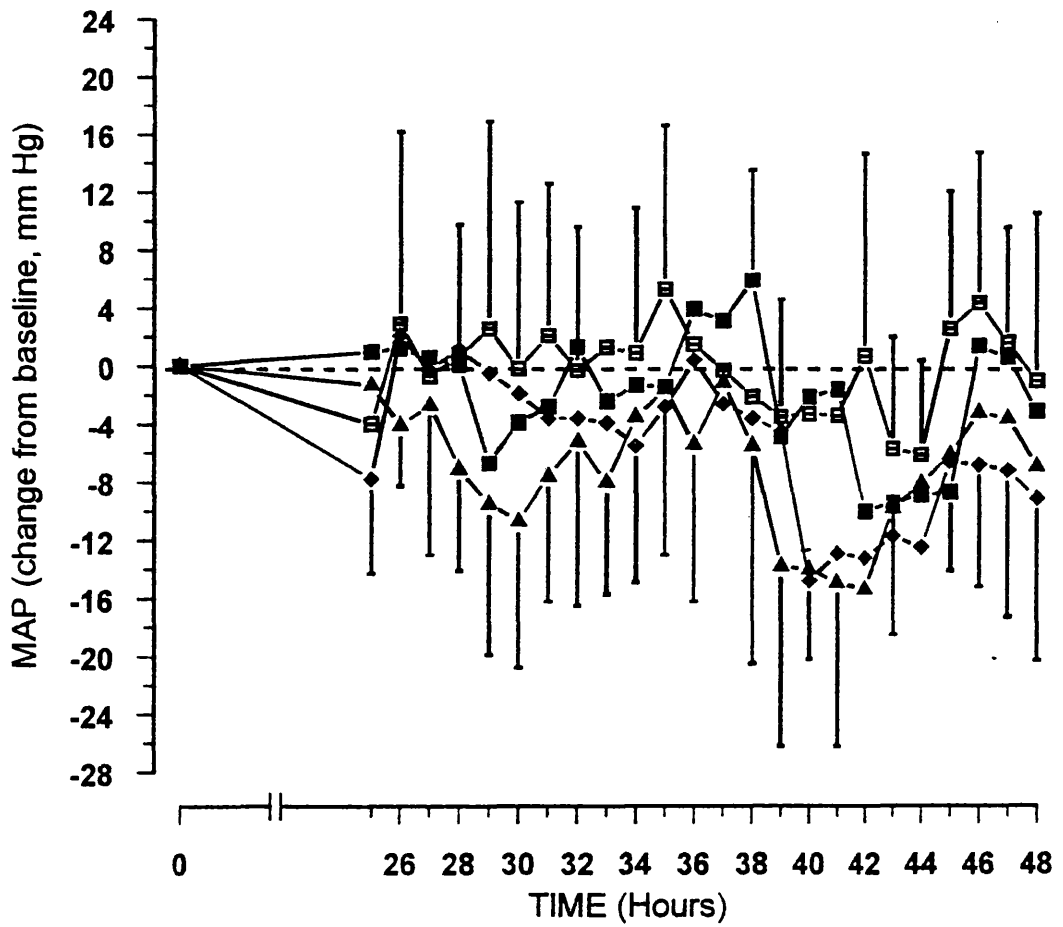
Figure 5.5:
Mean arterial pressure (change from baseline, mm Hg) following treatment
with placebo p.o.+ placebo p.o (□), enalapril 2.5mg p.o. + enalapril 2.5mg
p.o. (▲), perindopril 2mg p.o. + perindopril 2mg p.o. (■), or perindoprilat
0.167mg i.v. + perindopril 2mg p.o. (◇) in patients with congestive heart failure.
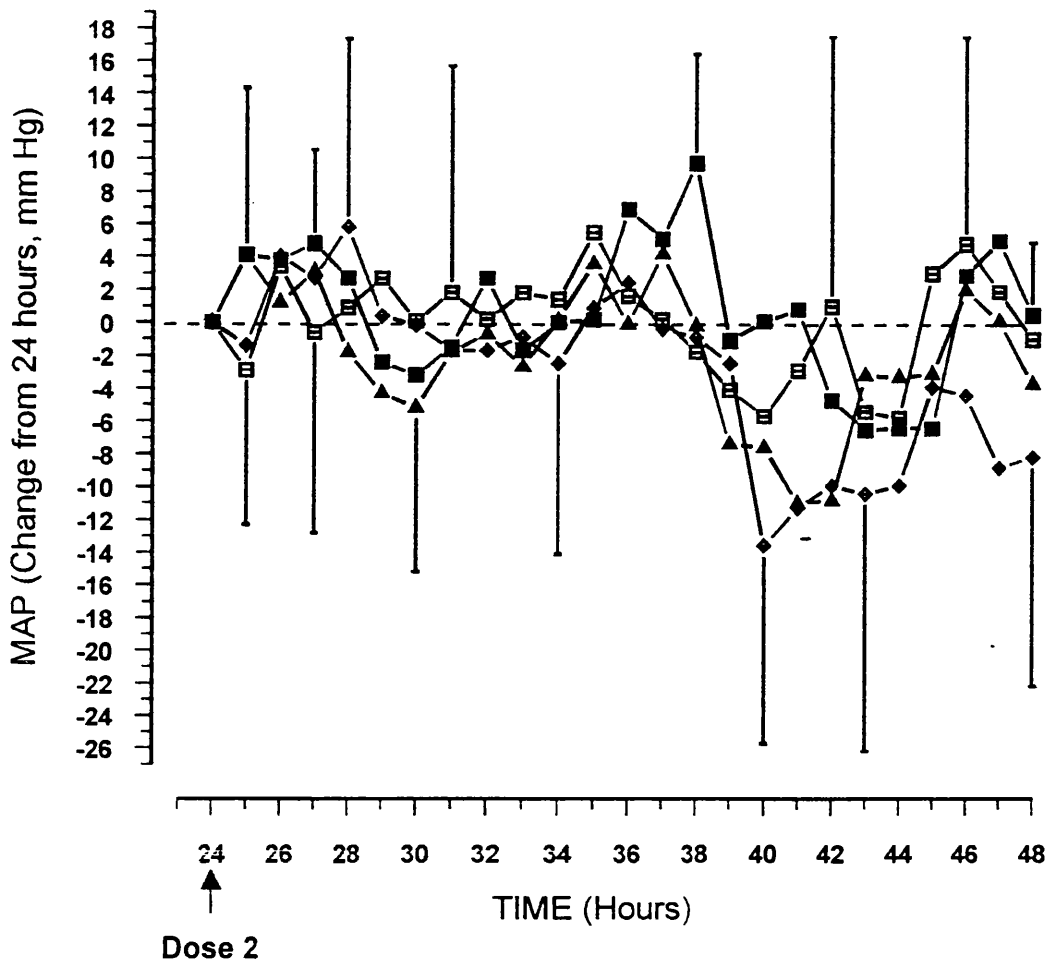
Figure 5.6:
Mean arterial pressure (change from 24-hour MAP, mm Hg) following
placebo p.o. + placebo p.o. (□), enalapril 2.5mg p.o + enalapril 2.5mg p.o. (▲),
perindopril 2mg p.o. + perindopril 2mg p.o., or perindoprilat 0.167mg i.v +
perindopril 2mg p.o. (◊) in patients with congestive heart failure.
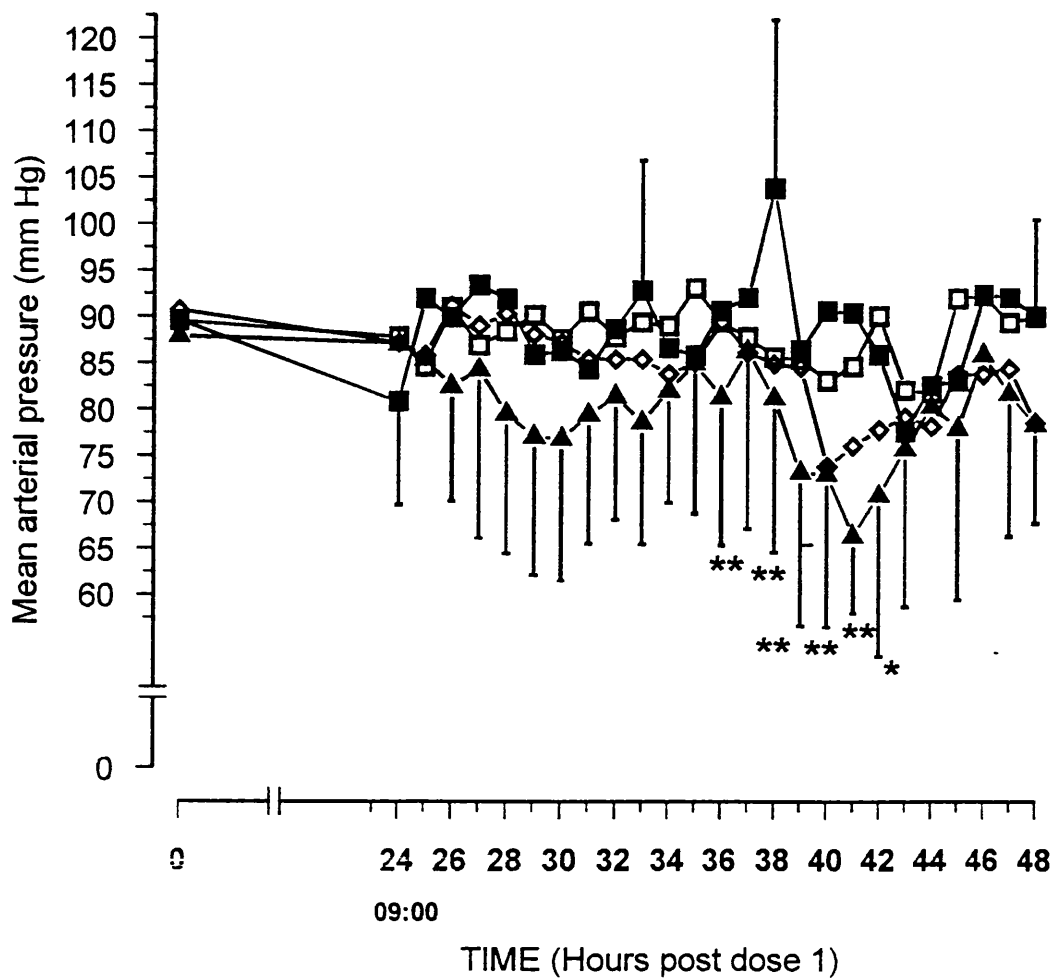
Figure 5.7:
Ambulatory mean arterial pressure (mm Hg) following placebo p.o.+
 placebo p.o. (□), enalapril 2.5mg + enalapril 2.5mg p.o. (▲), perindopril
2mg p.o.+ perindopril 2mg p.o.(■) or perindoprilat 0.167mg i.v. +
perindopril 2mg p.o.in patients with congestive heart
failure.
* p< 0.05 compared to placebo
** p< 0.05 compared to perindopril.

and diastolic blood pressures.

The change in blood pressure compared to blood pressure at 24 hours (i.e., prior to dose 2 of study medication) was also calculated for each treatment group. No significant change in MAP was found for any treatment group compared to placebo (Figure 5.6).

Comparison was made of the absolute values for MAP over day 2 of the study (Figure 5.7). Absolute MAP for the enalapril treatment group was consistently lower than for the other treatment groups and was statistically lower than for the perindopril group between 36 and 40 hours (21:00 - 02:00 on day 2) and from the placebo group at 42 hours (03:00 on day 2). Mean hourly ambulatory blood pressure was lower for enalapril (79.6mm Hg) than for other treatments (Perindopril 88.6, perindoprilat 84.2, placebo 88.0). Over day 2 of the study, mean hourly BP was lower for the group receiving perindoprilat as dose 1 plus perindopril as dose 2 than for those receiving perindopril or placebo as dose 1 (p<0.01), and greater than those receiving enalapril (p<0.01).

Comparison of the profiles of mean hourly systolic and diastolic blood pressures for perindopril and enalapril is shown in Figure 5.8. The mean hourly blood pressure profile for both systolic and diastolic blood pressure was consistently lower for the perindopril group than for enalapril. The difference was significant for diastolic blood pressure between 38 and 41 hours.

Heart rate response

Ambulatory heart rate showed marked fluctuation over the 24 hour period of the second study day (Figure 5.9). No differences were found among the treatment groups either in terms of the change in heart rate from the value at 24 hours or from baseline.
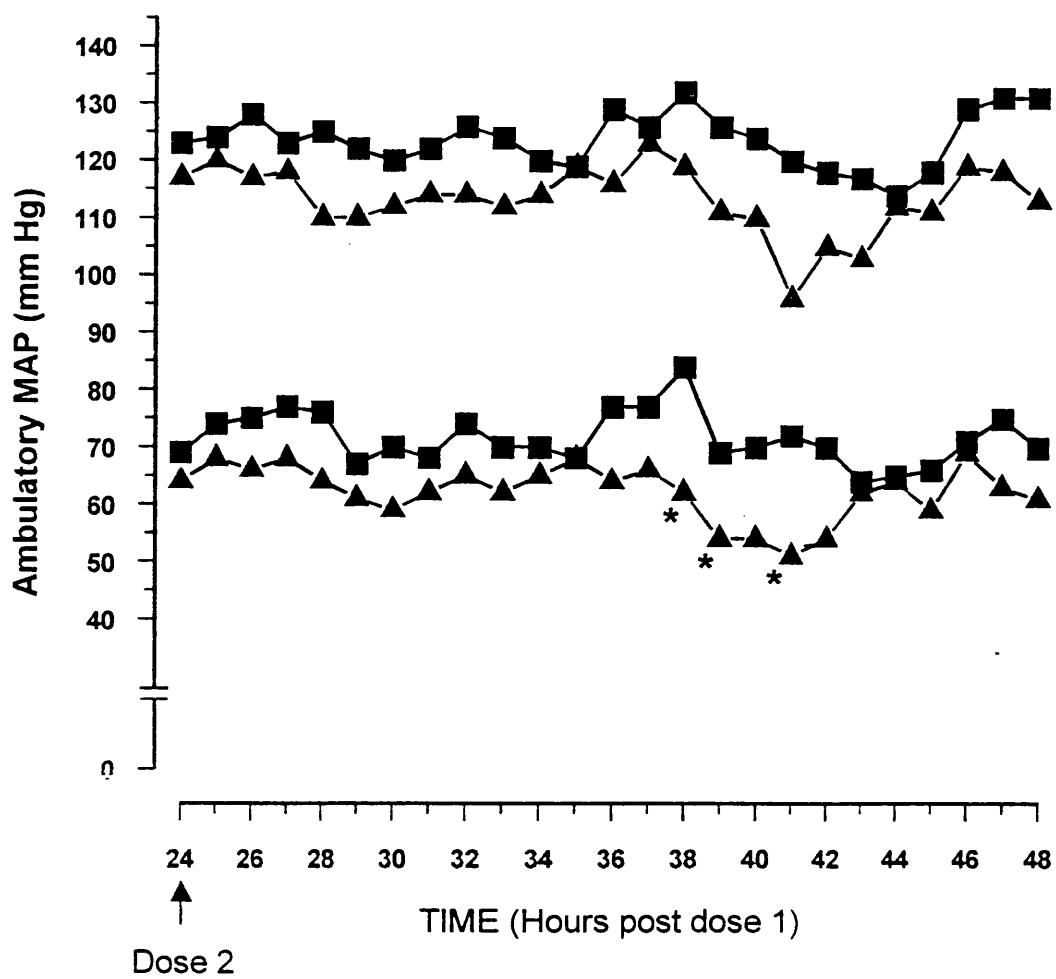
Figure 5.8:
Ambulatory mean systolic and diastolic blood pressure following two
doses of enalapril 2.5mg p.o. (▲) or perindopril 2mg p.o. (■) in patients
with congestive heart failure.
* p<0.05 cf perindopril
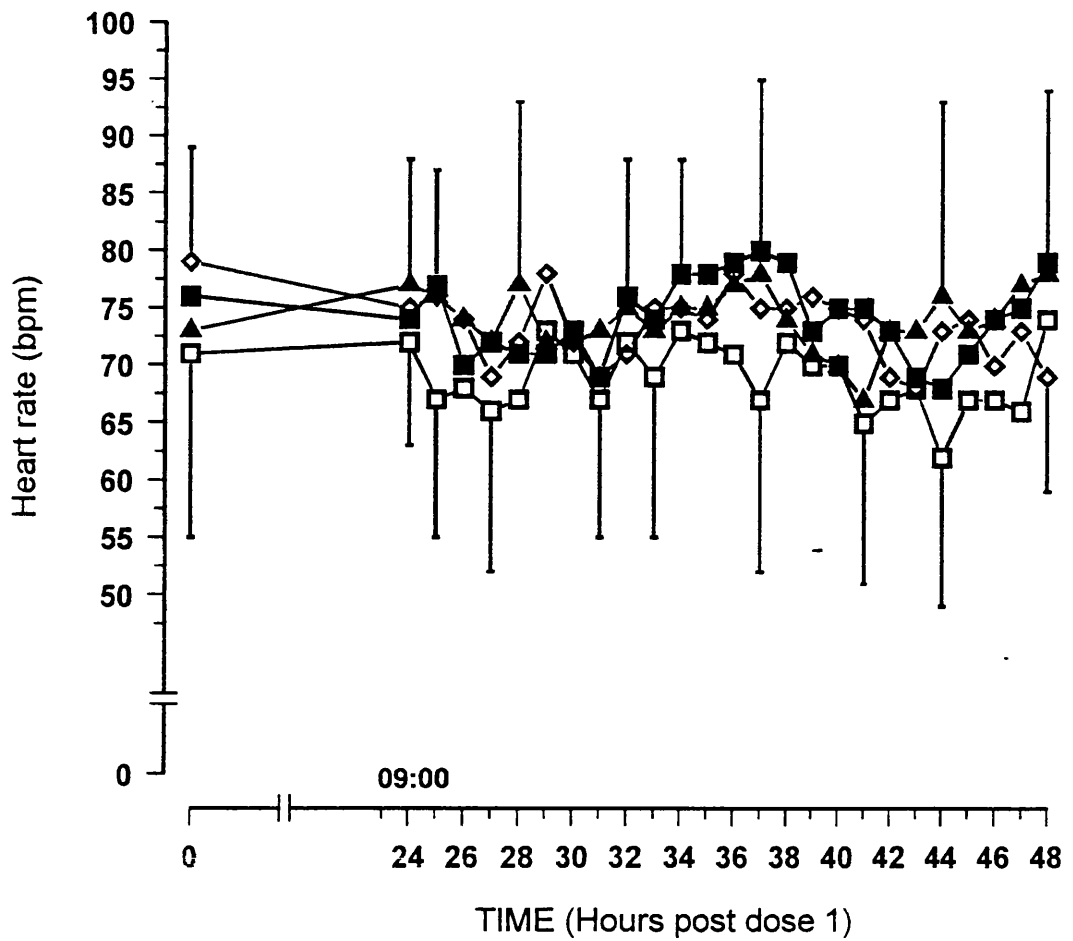
Figure 5.9:
Heart rate (bpm) following dose 2 of study treatment in patients with congestive heart failure.
Patients received placebo p.o. + placebo p.o.(□), enalapril 2.5mg p.o. + enalapril 2.5mg p.o., perindopril 2mg p.o. + perindopril 2mg p.o. (■) or perindoprilat 0.167mg i.v.+ perindopril 2mg p.o.(◇)

5.3.3 Drug concentrations

A. Enalapril 2.5mg p.o.

The pharmacokinetic profiles of enalapril and its metabolite enalaprilat were similar to those seen in previous studies in congestive heart failure, with an early peak concentration of the parent ester enalapril followed by a protracted plateau concentration of the diacid metabolite enalaprilat (Figure 5.10). The mean maximum concentration (C_{max}) of enalapril was 45.2 (20.5) ng/ml, (range 14.9-80.5). C_{max} of enalaprilat was 11.2 (5.7) ng/ml, (range 1.7-22.8). The mean time to peak concentration (T_{max}) of enalapril was 1.9 (0.8) hours; T_{max} of enalaprilat was 7.4 (2.0) hours.

Both enalapril and enalaprilat were detectable in the serum of all patients at 24 and at 48 hours after dose 1. At 24 hours after the first dose of enalapril 2.5mg p.o., mean enalapril concentration was 5.05 (5.69) ng/ml. At 48 hours after dose 1, i.e., after 2 doses of enalapril 2.5 mg p.o., enalapril concentration was 5.34 (6.98) ng/ml. Enalaprilat concentration at 24 hours was 3.86 (4.03) ng/ml. At 48 hours ,i.e., after 2 doses, enalaprilat concentration was 3.92 (3.94) ng/ml.

B. Perindopril 2mg p.o.

The pharmacokinetic profiles of perindopril and its active metabolite perindoprilat were similar to those seen in previous studies in congestive heart failure. Profiles were qualitatively similar to those for enalapril and enalaprilat with an early peak of parent ester followed by a prolonged plateau of diacid metabolite (Figure 5.11). The mean maximum concentration (C_{max}) of perindopril was 32.2 (16.0) ng/ml (range 9.8-67.1). C_{max} for perindoprilat was 4.10 (1.65) ng/ml (range 2.1-7.7). The mean time to peak perindopril

133

concentration (T_{max}) was 2.0 (0.9) hours (range 1.25-4.0); T_{max} for perindoprilat was 5.1 (3.0) hours (range 0.83-10). The diacid metabolite perindoprilat was detectable at 24 hours in all 12 subjects receiving perindopril 2mg p.o on day 1. Mean plasma perindoprilat concentration was 1.9 (1.6) ng/ml at 24 hours. Low levels of parent ester remained detectable in 10 of 12 subjects at 24 hours (mean concentration 1.9 ng/ml, range 0-1.8). At 48 hours after dose 1, perindopril was detectable in the plasma of 9 of the 10 subjects for whom a drug concentration was obtained at this time. The mean area under the perindoprilat concentration-time curve (AUC) from 0-24 hours was 60.14 (29.46) ng/ml.hr., significantly higher than seen with the intravenous infusion (p=0.001).

C. Perindoprilat 0.167mg i.v.

The pharmacokinetic profile following constant rate i.v. infusion of 0.167mg perindoprilat is shown in Figure 5.12. Perindoprilat concentration reached a peak at the end of the 1 hour infusion period. Following termination of the i.v. infusion, perindoprilat concentration declined slowly over the 24 hour period. Mean maximum (C_{max}) perindoprilat concentration was 4.4 (2.9) ng/ml (range 1.5-9.9).

T_{max} was 0.96 (0.14) hours (range 0.67-1.25). Perindoprilat was detectable in all 12 subjects at 24 hours, mean concentration at this time being 0.63 (0.38) ng/ml (range 0.1-1.5). Mean perindoprilat concentration at 48 hours was 1.64 (0.89) ng/ml (range 0-2.8). Drug was detectable in the serum 9 of the 10 patients for whom a serum sample was obtained at this time. The mean AUC (0-24 hrs) for perindoprilat was 23.59 (12.64) ng/ml.hr, significantly lower than seen with perindopril 2mg p.o.(p=0.001).
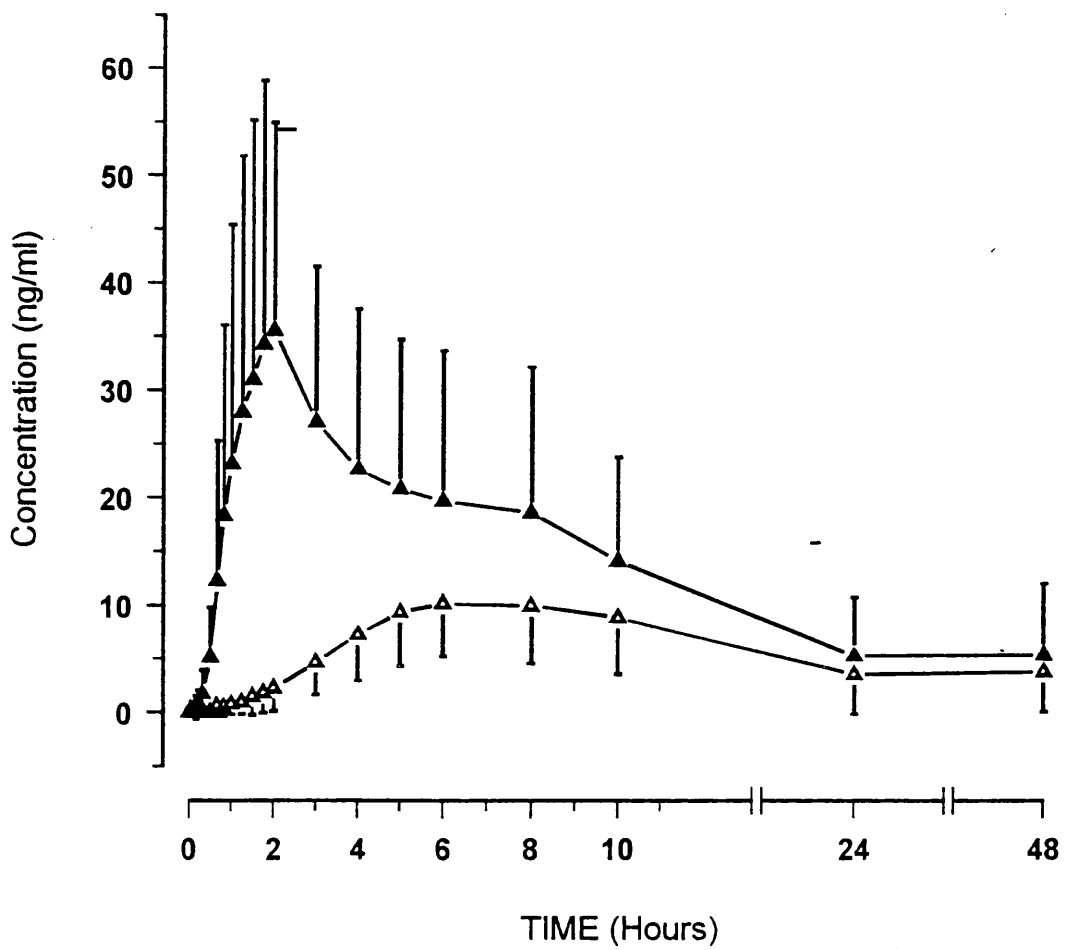
Figure 5.10:
Plasma concentration (Mean±1 SD) of enalapril (▲) and enalaprilat (△)
after oral administration of enalapril 2.5mg p.o. in patients
with congestive heart failure.
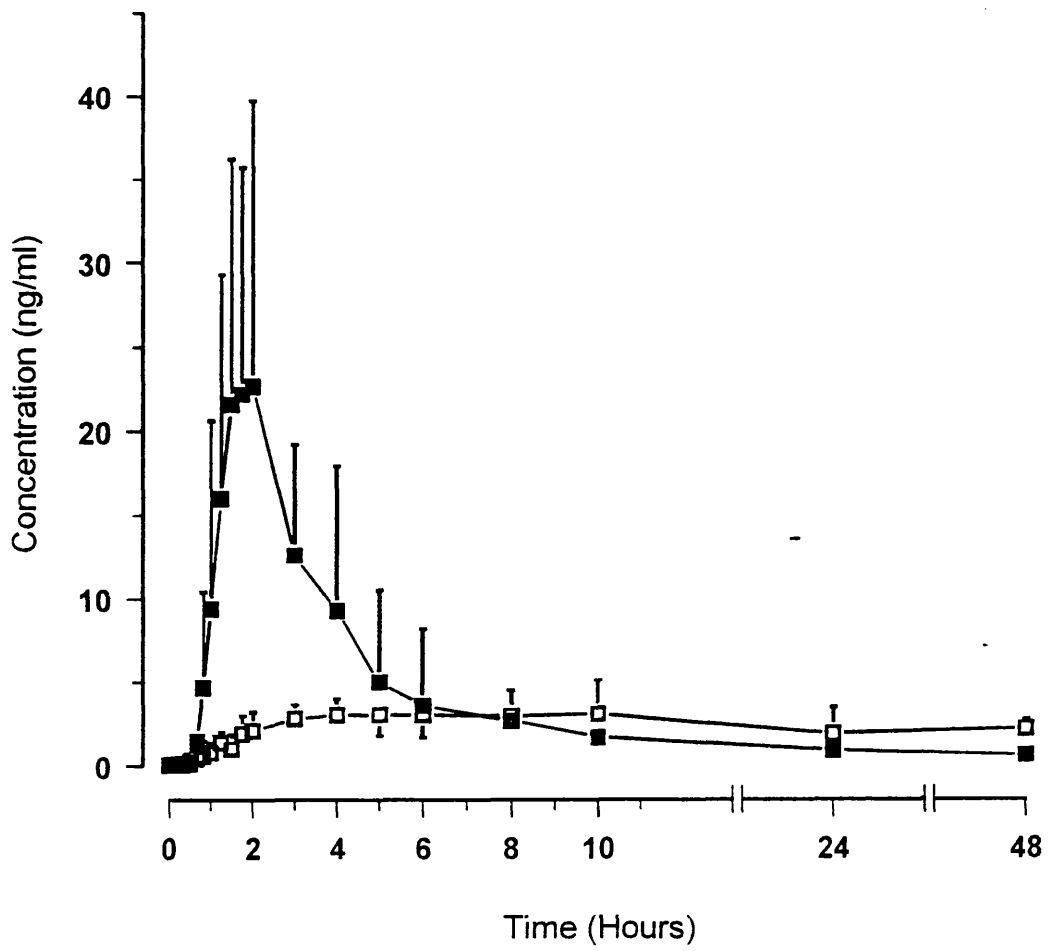
Figure 5.11:
Plasma concentration (Mean ± 1SD) of perindopril (■) and perindoprilat (□)
after oral administration of perindopril 2mg in patients with heart failure.
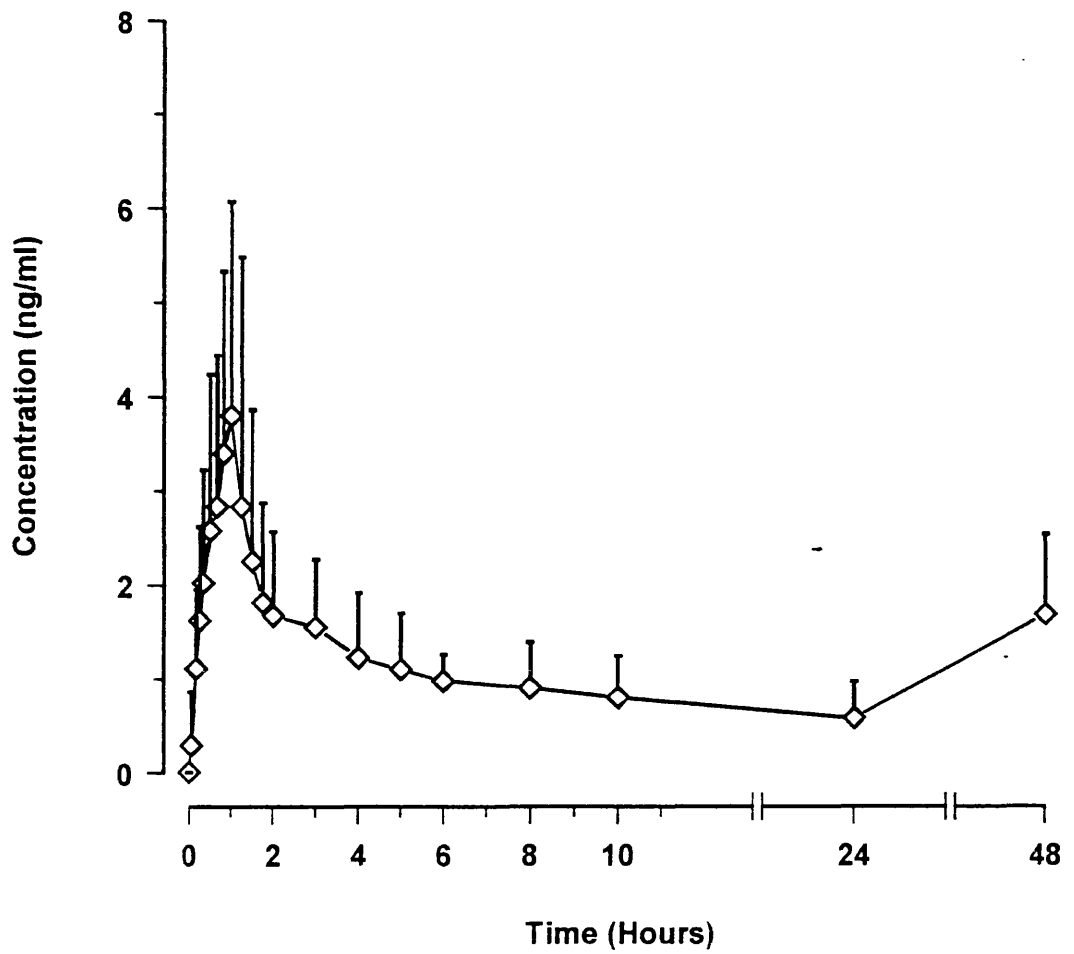
Figure 5.12:
Plasma concentration of perindoprilat (◇)(Mean ± 1SD) following infusion of perindoprilat 0.167 mg i.v. over 1 hour in patients with congestive heart failure.

5.4 DISCUSSION

5.4.1 1st dose haemodynamic responses

Blood pressure response

The present study confirms disparate blood pressure responses to the first dose of the pro-drug ester ACE inhibitors perindopril 2mg p.o and enalapril 2.5mg p.o. in elderly patients with congestive heart failure. As in the previous study (MacFadyen et al 1991b), similar profiles of plasma ACE inhibition were seen with these doses. Quantitatively, enalapril showed slightly greater maximal inhibition of plasma ACE than perindopril, the reverse of the pattern seen in the previous study. The transient pressor response observed after intravenous dosing with perindoprilat in the previous study was not observed. A small, transient rise in MAP was observed after oral dosing with perindopril and with placebo but did not attain statistical significance. This trend was paralleled by a rise in heart rate over the first hour of the study with all oral therapies including placebo. In contrast to a previous study (MacFadyen et al 1993) these effects were not seen with intravenous perindoprilat. Although it is possible that the pressor response and increase in heart rate are due to displacement of bound angiotensin II, it is perhaps more likely that they simply reflect the stress associated with the start of the study, a period of intensive blood pressure monitoring and blood sampling. The tendency for these phenomena to be seen after dosing with placebo would tend to support this explanation.

Demographic differences among patient groups do not apear to explain the observed differences in haemodynamic profile. Groups were well matched in terms of NYHA class, diuretic dose and laboratory parameters. Similarly, the aetilogy of CHF does not explain the differences (see Chapter 8). In particular, in all 4 patients in whom valvular disease was felt to be contributory to CHF the lesions were regurgitant in nature and

differences in this regard among treatment groups are most unlikely to explain the observed haemodynamic differences.

Heart rate response

Significant reductions in mean supine heart rate were seen following perindopril 2mg p.o. and perindoprilat 0.167mg i.v. In contrast to previous observations (MacFadyen et al 1991) no fall in heart rate was seen following enalapril 2.5mg p.o. There is no obvious explanation for the diversity of findings in this respect between the two studies. The results confirm the lack of reflex tachycardia associated with the commencement of ACE inhibitor therapy in CHF. One possible explanation for the reduction in heart rate following commencement of ACE inhibitor therapy would be an effect on the autonomic nervous system. There is abundant evidence of a synergystic interaction between the renin-angiotensin system and the sympathetic nervous system (Squire and Reid 1993). Inhibition of ACE may theoretically reduce sympathetic activity and thus reduce heart rate. Alternatively, a parasympathomimetic effect of ACE inhibitors has been postulated (Ajayi et al 1985). There is no direct evidence for either effect from this study.

5.4.2 Blood pressure response 10-48 hours after initiation of therapy.

The haemodynamic response over the first 10 hours following initiation of therapy differed between perindopril and enalapril. At 24 hours, MAP had fallen by a mean of approximately 9mm Hg in each group compared to baseline. Neither the change in MAP nor the absolute MAP at this time differed between the groups. At this point, 24 hours after the first dose of oral therapy, MAP appears to be falling in the perindopril group but rising in the enalapril group. The question as to whether perindopril is associated with a blood pressure fall between 10 and 48 hours after initiation of therapy has only been

partially answered by the present study. Measured ambulatory blood pressure responses following the second dose of oral therapy were very variable and no difference between perindopril and enalapril groups could be identified in terms of the fall in ambulatory MAP. The results demonstrate that the mean hourly ambulatory blood pressure after the second dose of oral therapy was lower in those patients receiving enalapril compared to those receiving perindopril.

The present study has confirmed that the mean fall in MAP after perindopril 2mg p.o. is not different to that seen after placebo. Although the administration of perindoprilat 0.167mg i.v. over 1 hour also showed no effect on blood pressure compared to placebo, the trend in MAP over the period of infusion is unequivocally down as compared to the placebo response over the same period. If we postulate that the BP response is a phenomenon related to plasma drug concentration, intravenous infusion of 0.4mg perindoprilat (an amount of perindoprilat equivalent to 2mg perindopril p.o. assuming 20% oral bioavailability of perindopril as perindoprilat) would have been more likely to elicit a fall in MAP. The greater mean area under the concentration-time curves (0-24 hrs) indicate that the oral dose provided a greater total amount of drug than intravenous perindoprilat. Intravenous doses of perindoprilat of 1mg lower blood pressure in patients with CHF (MacFadyen et al 1993).

5.4.3 Pharmacokinetic profiles

It is of interest to compare the relative haemodynamic and pharmacokinetic profiles following perindopril 2mg p.o. and perindoprilat 0.167mg i.v. The C_{max} for perindoprilat was the same after perindopril 2mg p.o. (4.1 ng/ml) as after perindoprilat 0.167mg i.v. The maximum mean fall in MAP after perindoprilat i.v. (7.8mm Hg) was seen at the end of the 1 hour infusion period. Mean plasma perindoprilat concentration at this

time was 3.68 ng/ml. This compares with a maximum mean fall of 6.8mm Hg seen at 4.5

hours following perindopril p.o. The concentration of perindoprilat at this time was

approximately 3 ng/ml. It is of note however that in the group receiving perindoprilat i.v.,

mean BP fall at this time, i.e., 4.5 hours post dose, was 6.7mm Hg and circulating

perindoprilat concentration approximately 1.3 ng/ml. There appears therefore to be some

discrepancy between the two treatments in terms of the relationship of haemodynamic

response to plasma drug concentration. Overall the pharmacokinetic profiles of all active

treatments were broadly comparable to those seen with the same doses in previous studies.

CHAPTER 6

THE HAEMODYNAMIC AND NEUROHORMONAL RESPONSE TO THE

FIRST DOSE OF QUINAPRIL IN CHF

6.1 INTRODUCTION

In recent years a number of ACE inhibitor agents have been introduced for the treatment of CHF. A variety of claims have been made for the relative merits of each of these, such as preferential binding to tissue or cardiac ACE. The relative efficacy of the agents available in terms of improvement in mortality and morbidity in CHF has not been addressed and is unlikely to be so in the near future.

Quinapril is a recently introduced, non-sulphydryl ACE inhibitor. Previous reports associated quinapril with a lower incidence of first-dose hypotension than either enalapril or captopril (Wadworth & Brogden 1991). Moreover, this agent has been suggested to have a relatively higher affinity for tissue ACE (Nakajima et al 1992). A recent study showed that relatively greater inhibition of tissue ACE is produced bt a single 20mg dose of quinapril compared to the same dose of enalapril at a time when inhibition of plasma ACE is equivalent (Lyons et al 1997). Given that the relationship of the fall in BP to the level of plasma ACE inhibition is unclear, and that this response may be dependent upon inhibition of tissue ACE, this study set out to compare the first-dose haemodynamic response to the first dose of the prodrug ACE inhibitor quinapril in elderly patients with CHF. The currently recommended starting dose of the agent in heart failure was chosen for comparison with placebo on a background of supervised diuretic withdrawal. The main points of interest were to be the haemodynamic and neurohormonal responses and the pharmacokinetic profile following a single oral dose of quinapril 2.5 mg.

6.2 PATIENTS AND METHODS

This study was carried out in patients with chronic stable congestive cardiac failure (n=24, 54-88 years; 11:13, M:F), New York Heart Association (NYHA) functional class II-IV. All patients were symptomatic on stable doses of diuretic therapy prior to the study. All patients were admitted to hospital for commencement of ACE inhibitor therapy. All had erect systolic blood pressure of > 100 mm Hg, and none had significant disturbance of renal or hepatic function, prior to the study.

The diagnosis of CHF was confirmed by clinical history and by physical, ectrocardio-graphic and echocardiographic examination in all cases. A number of patients had also undergone radionuclide scanning or cardiac catheterisation. Demographic details of patients receiving placebo and quinapril are shown in Tables 6.1 and 6.2 respectively. In all but one patient, all diuretic therapy was withdrawn for 48 hours prior to the study day. In this one individual (patient no. 13), diuretic withdrawal was for 24 hours due to concerns regarding stability of the clinical condition. All vasodilator (eg oral nitrate, calcium channel blocker) or ß-blocker (patient 24 only) therapy was witheld on the day of the study; digoxin, where prescribed, was continued. On the morning of treatment, the individual patient rose, washed and had breakfast as normal. The patient then returned to bed where they rested supine. A peripheral venous cannula was inserted in an antecubital fossa vein for the purpose of blood sampling.

Following 45-60 minutes supine rest, blood was drawn for estimation of baseline drug concentration, ACE activity, plasma renin activity, aldosterone and angiotensin II levels. Blood pressure was measured at 2 minute intervals during this period using a semi-automatic sphygmomanometer (Sentron, Bard, Sunderland, U.K.) to establish baseline values. Baseline blood pressure values represent the mean of the recordings at 2 minute

Table 6.1

Demographic data
Placebo

Study No.	Age (years)	Sex	Aetiology	NYHA Class
3	56	F	IHD	II
5	77	M	IHD	II
8	85	F	IHD/HT	III
9	60	M	IHD/HT	III
12	69	M	IHD	II
13	55	F	IHD/HT	II
15	82	F	IHD	IV
17	68	M	IHD	II
20	76	F	IHD	III
21	73	M	IHD/HT	III
23	74	F	IHD	III

69.1 ± 10.7

4 atrial fibrillation/ 8 sinus rhythm

Frusemide 70 ± 27 mg/day

IHD	Ischaemic heart disease
HT	Hypertension
NYHA	New York Heart Association functional class

Table 6.2

Demographic data
Quinapril 2.5 mg

Study No.	Age (years)	Sex	Aetiology	NYHA Class
2	79	F	IHD/HT	II
4	67	M	IHD	II
6	88	F	IHD	II
7	81	F	HT	III
10	75	M	IHD/HT	II
11	85	F	IHD/HT	III
14	68	F	IHD	II
16	61	F	IHD/HT	III
18	76	M	IHD	II
19	74	M	IHD/HT	II
22	83	F	IHD/HT	II
24	72	M	IHD	II

75.6±8.2

5 atrial fibrillation/ 7 sinus rhythm

Frusemide 56.7±27 mg/day (p=0.42)

IHD	Ischaemic heart disease
HT	Hypertension
NYHA	New York Heart Association functional class

intervals during the final 30 minutes of this period. Oral study medication (quinapril 2.5 mg or matching placebo) was then administered in a double-blind fashion according to the randomisation schedule held by the hospital Department of Pharmacy. Blood sampling and triplicate determination of blood pressure were carried out at set time points until 10 hours after administration of the study drug. Patients remained supine until this time, at the end of which they were allowed to resume normal activity. Blood sampling and blood pressure measurements were repeated at 24 hours, prior to which the patients again rested supine for 45-60 minutes.

At the end of the 24 hour study period the treatment code was broken by the Department of Pharmacy. Patients who had received active therapy were continued on open ACE inhibitor therapy (mostly enalapril 5 mg b.d.) while those who had received placebo were given a standard oral test-dose of captopril 6.25 mg. Patients receiving placebo were not included in any other study phase. Vasodilator and diuretic therapies were reintroduced as required. Blood sampling for routine haematological and biochemical estimation was carried out prior to the commencement of the study and at the end of the 24 hour study period.

6.3 RESULTS

6.3.1 General

The patients in each study group were well matched in terms of age, severity of CHF and diuretic dosage (Tables 6.1 & 6.2). Pre-treatment haemoglobin concentration was higher in the placebo group (p=0.04). None of the biochemical parameters was significantly altered 24 hours after initiation of therapy, either active or placebo (Table 6.3). A small fall of approximately 1g/dl was seen in haemoglobin concentration and is

Table 6.3

Laboratory data. Baseline values and change over study period (Mean (1SD))

	Quinapril	Placebo	p-value
Sodium (mmol/L)	140.7 (3.1)	140.5 (1.9)	0.91
Sodium (change)	-3.2 (3.3)	-0.4 (3.5)	0.07
Potassium (mmol/L)	4.0 (0.4)	4.4 (0.4)	0.10
Potassium (change)	+0.3 (0.4)	+0.2 (0.5)	0.39
Urea (mmol/L)	9.5 (4.4)	8.1 (3.7)	0.39
Urea (change)	-0.5 (1.6)	-0.9 (1.4)	0.39
Creatinine (μmol/L)	126.7 (52.4)	114.3 (30.2)	0.42
Creatinine (change)	-0.8 (27.3)	-10.8 (17.3)	0.3
Haemoglobin (g/dL)	12.8 (1.5)	14.3 (1.8)	0.04
Haemoglobin (change)	-1.0 (0.8)	-1.1 90.4)	0.82
Baseline PRA (ng AI/ml/Hour) (Mean(95% C.I.))	0.86 (1.21-3.39)	1.85 (1.20-6.81)	0.10
Baseline aldosterone (pg/ml)	111 (66)	155 (118)	0.28

Table 6.4

Patient characteristics. Baseline supine blood pressure and heart rate (Mean (1SD))

	Quinapril	Placebo	p-value
Systolic BP (mm Hg)	142 (18)	125 (20)	0.055
Diastolic BP (mm Hg)	72 (9)	69 (17)	0.55
Mean BP (mm Hg)	95 (12)	88 (16)	0.21
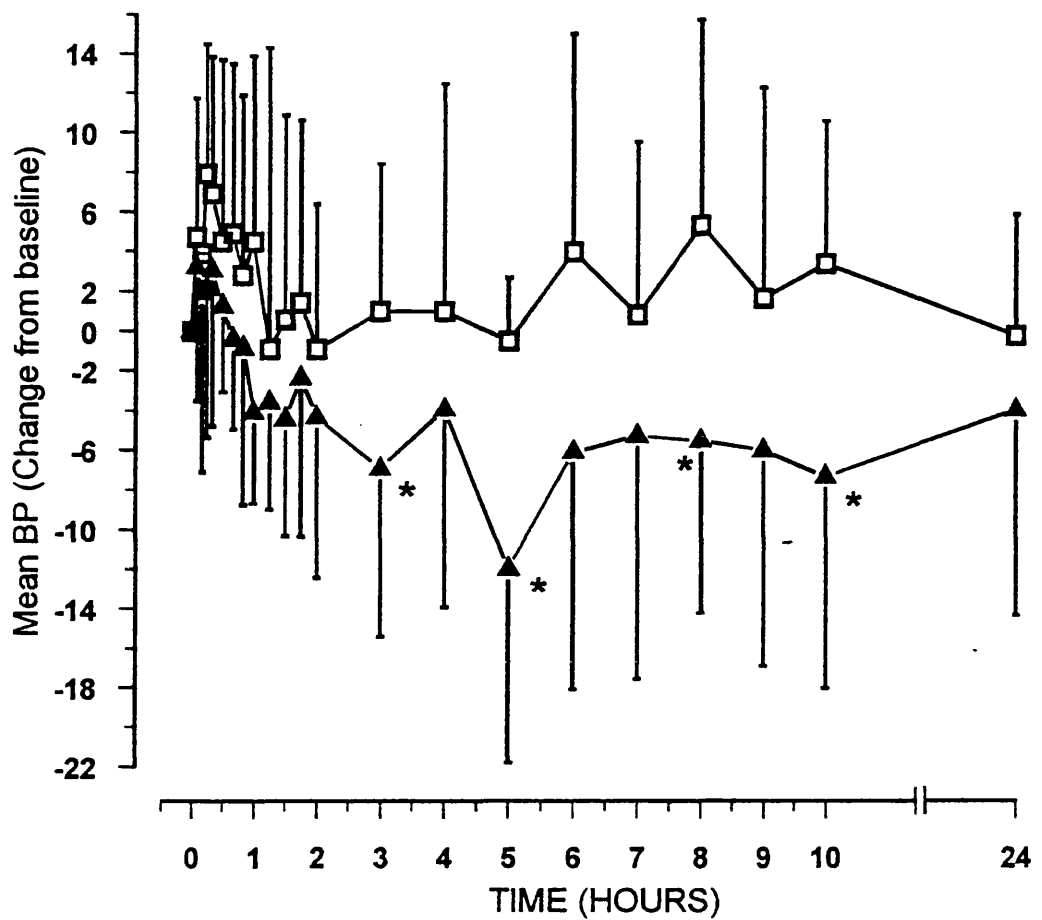
Figure 6.1 :
Baseline corrected change in mean arterial blood pressure (Mean±1SD) after quinapril 2.5mg p.o. (▲) or placebo (□) in patients with congestive heart failure.
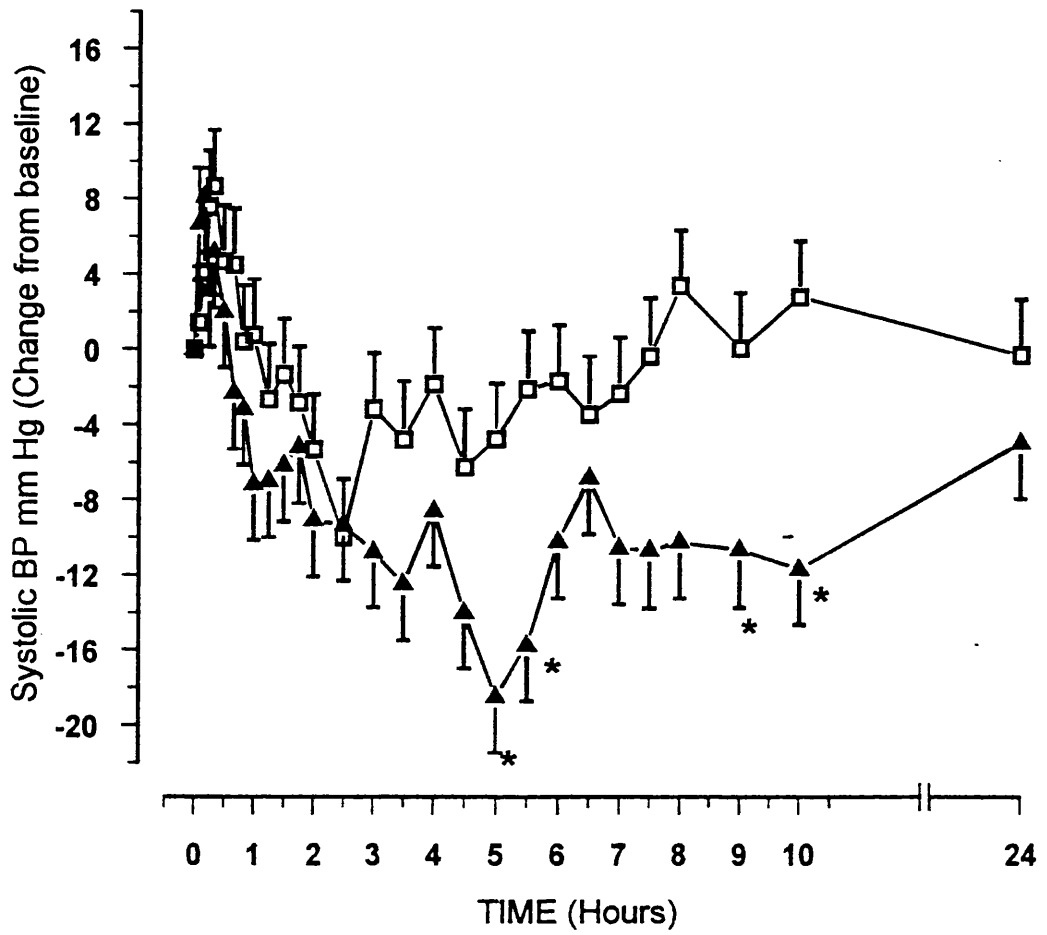
Figure 6.2:
Baseline corrected change in systolic blood pressure (Mean±1 S.D.)
following quinapril 2.5mg p.o.(▲)or placebo (□) in patients with
congestive heart failure.
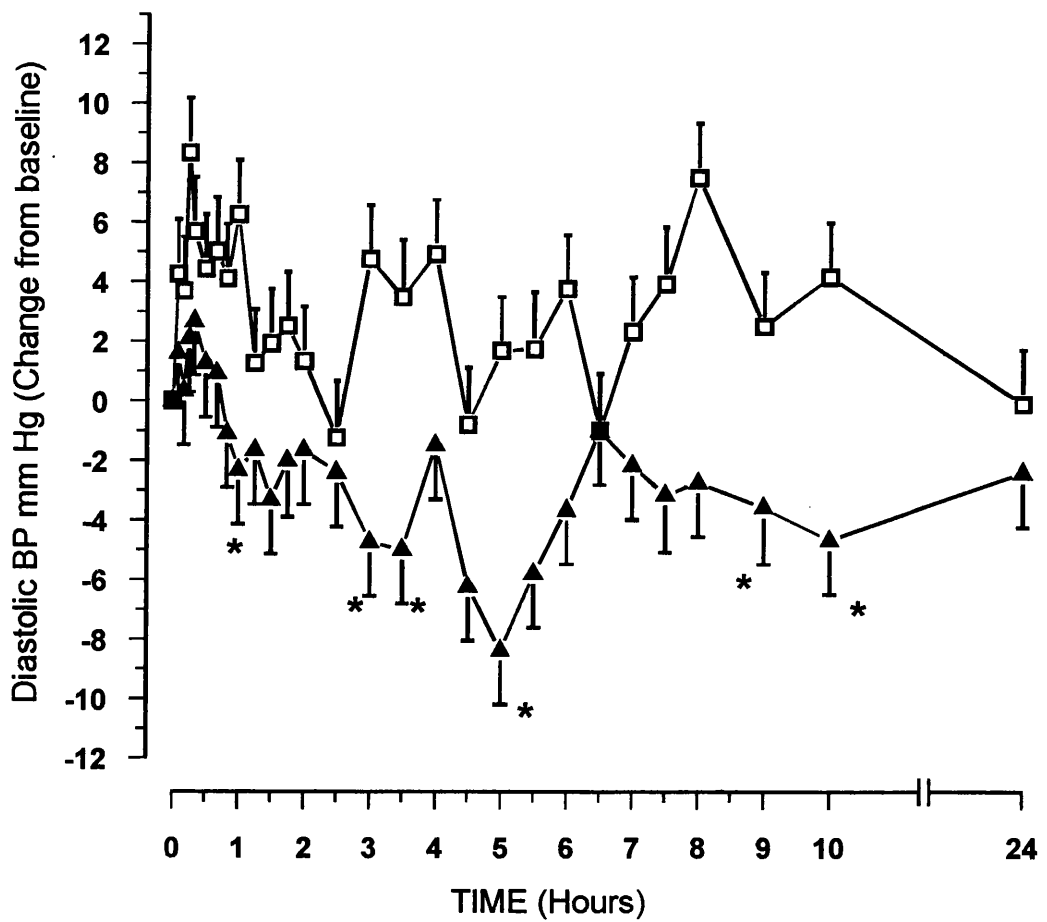
Figure 6.3:
Baseline corrected change in diastolic blood pressure (Mean±1 S.D.)
following quinapril 2.5mg p.o.(▲) or placebo (□) in patients with
congestive heart failure.

attributable to blood sampling. All patients remained asymptomatic during supervised diuretic withdrawal and during the study period. One patient receiving active therapy (Subject 18) showed a rise in serum creatinine concentration, from 196μmol/l prior to treatment to 242μmol/l 24 hours following acute ACE inhibition. This rise was transient and did not necessitate discontinuation of maintenance therapy.

There were three adverse events during the study, none of which was felt to be directly related to study medication. One patient who had received placebo therapy (Subject 23) was unable to tolerate maintenance ACE inhibitor therapy due to symptomatic hypotension following the first dose of oral captopril 6.25 mg. The post-study ECG of a second patient who had received placebo therapy (Subject 21) showed anterolateral ST segment depression without associated symptoms. The patient had known stable ischaemic heart disease and no further action was required. One patient who had received active therapy (Subject 11) developed a pyrexia during the study period; a urinary tract infection was diagnosed clinically and confirmed on urine culture.

6.3.2. Haemodynamic response

Blood pressure and heart responses at each time point are described as change from baseline values. Pretreatment baseline MAP was similar in treatment groups (Table 6.4). Systolic blood pressure tended to be higher (p=0.055) in the group receiving quinapril (141.8±18 mm Hg) compared to those receiving placebo (125.0±20). Baseline heart rate was similar in the two groups (Table 6.4).

Blood pressure

In both active and placebo groups, a transient rise in blood pressure was observed during the early part of the study period immediately following administration of the study
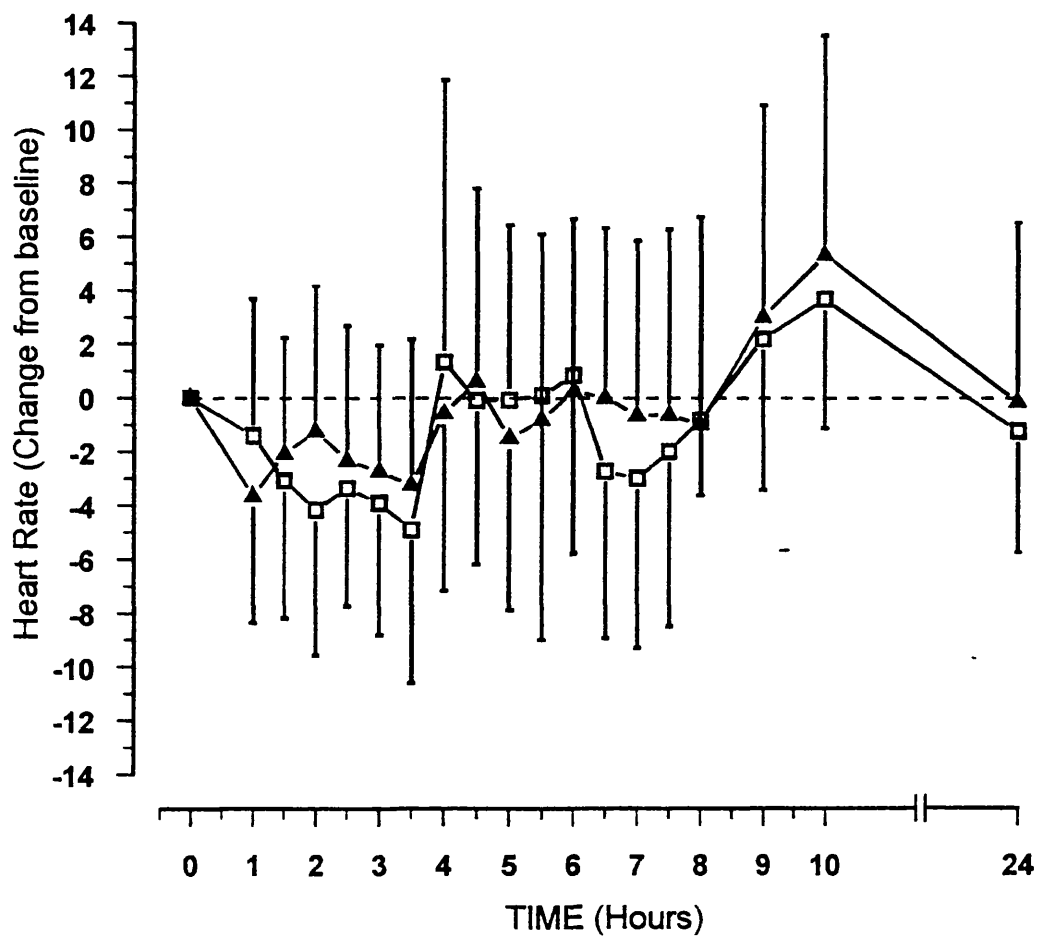
Figure 6.4 :
Change in heart rate (mean±1SD)(change from baseline, bpm) after
quinapril 2.5mg p.o. (▲) or placebo (□) in patients with congestive
heart failure.

drug. There was no statistical difference between the groups in the magnitude of this blood pressure change. Following this early rise in MAP, quinapril lowered blood pressure compared to placebo. The magnitude of the blood presure fall was greater in absolute terms for systolic blood pressure than for diastolic or mean blood pressure. For MAP, the change from baseline was significant compared to placebo at 3, 5, 8 and 10 hours (Figure 6.1). For systolic blood pressure, the change was significant at 5, 5.5, 8 and 10 hours (Figure 6.2). For diastolic blood pressure, the change was significant at 1, 3, 3.5, 5, 8 and 10 hours (Figure 6.3).

The maximum mean fall in MAP compared to baseline was 12.0 (9.8) mm Hg, seen at 5 hours post dose. The mean maximum fall in MAP for individual subjects, irrespective of its time course, was 19 (8) mm Hg in the quinapril group (range 0 to 27 mmHg) at 5.1 (2.9) hours and 13 (13) mm Hg at 7.42 (8.10) hours in the placebo group (range 0 to 42 mmHg) (p=0.20, N.S.)

Heart rate

Supine heart rate showed only minor fluctuations over the period of study. No significant change from baseline value was seen at any time point for either active or placebo therapy. Heart rate did not change after initiation of therapy with quinapril (Figure 6.4).

6.3.3 Plasma ACE inhibition

Plasma ACE activity was rapidly inhibited in the active treatment group. Inhibition reached a statistically significant level by 50 minutes after drug administration. Plasma ACE inhibition was maximal at 3 hours (87.2 ± 6.2%) although there was no statistical difference between the degree of inhibition seen at any time point between 2 and 10 hours.

The degree of ACE inhibition at 24 hours in the active treatment group remained

significant (61.3 ± 20.2%) compared to baseline (Figure 6.5).

6.3.4 Plasma drug concentration

Concentrations of both quinapril and the active metabolite quinaprilat were

determined at frequent intervals during the study day (Figure 6.6). The mean time(SD) to

peak concentration (T_{max}) for quinapril was 2.56 (1.15) hours while the mean time to peak

quinaprilat concentration was 3.56 (0.84) hours. The mean maximum concentration (C_{max})

of quinapril was 49.7 (30.9) ng/ml (range 25.2-138.5). The mean maximum concentration

of quinaprilat was 51.0 (22.8) ng/ml (range 29.4-103.5). The active moiety quinaprilat was

detectable in the plasma within 30 minutes, and remained detectable at 24 hours, in all 12

subjects receiving active therapy (mean 4.4±5.2 ng/ml, range 1.5-20.3). Mean quinapril

concentration (n=12) was 1.8 (2.2) ng/ml (range 0.0-7.1) at 24 hours. Quinapril was

undetectable at this time in 3 of 12 subjects.

The half-life ($t_{1/2}$) estimated by log linear regression analysis of plasma quinaprilat

concentrations at 8, 10 and 24 hours was 6.1 hours. The terminal half-life could not be

estimated in view of the short sampling period.

6.3.5 Plasma renin activity

Plasma renin activity (PRA) at baseline showed wide interindividual variation.

Baseline PRA was higher in the placebo group (p=0.10) (Table 6.3). Plasma renin activity

in the active treatment group showed the expected rise compared to placebo.

Plasma renin activity (PRA) at baseline showed wide interindividual variation.

Baseline PRA was higher in the placebo group (p=0.10) (Table 6.3). Plasma renin activity

in the active treatment group showed the expected rise compared to placebo (Figure 6.7).
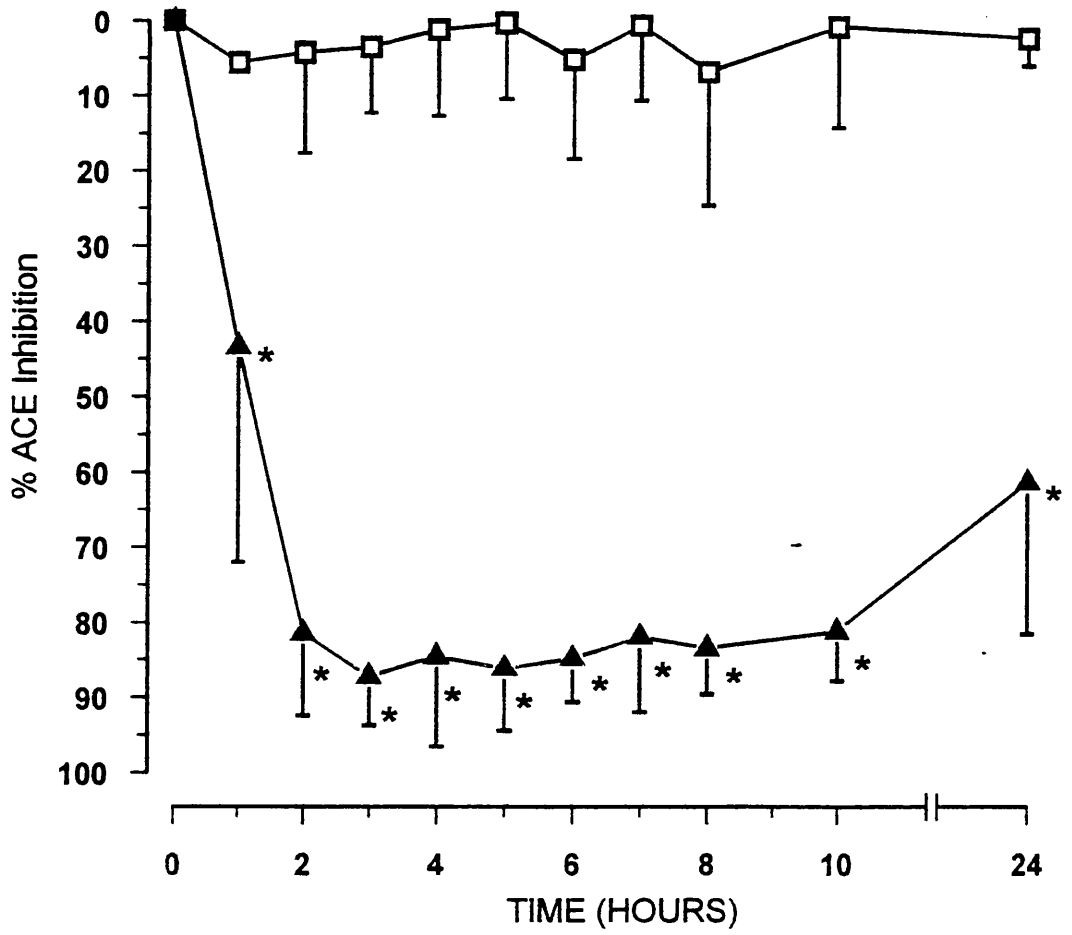
Figure 6.5:
Plasma ACE inhibition (% baseline activity)(Mean ± 1SD) after quinapril
2.5 mg p.o.(▲) or placebo (□) in patients with congestive heart failure.
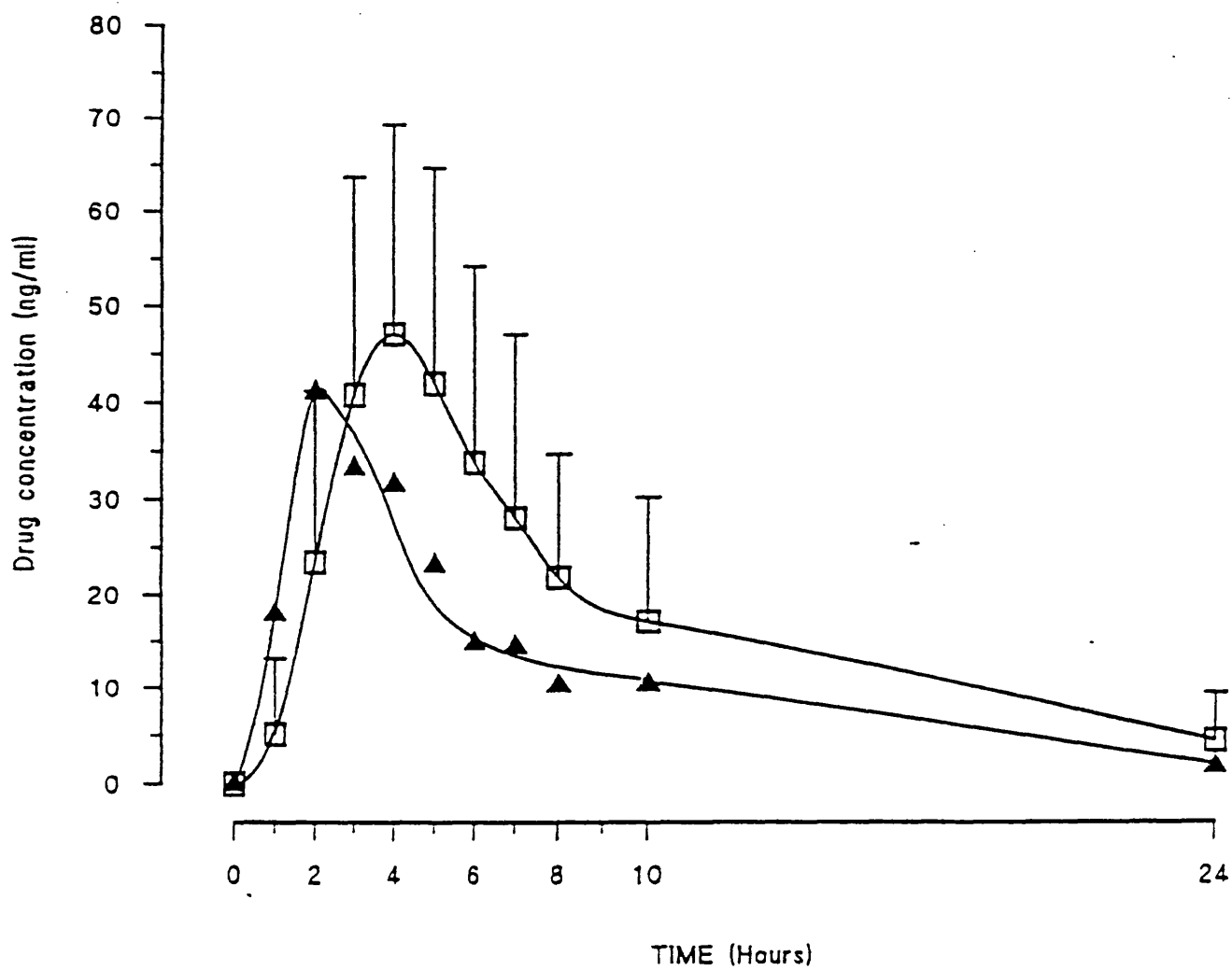
Figure 6.6:

Plasma concentrations of quinapril (▲) and quinaprilat (□) following a single dose of quinapril 2.5mg po in patients with CHF.

In relative terms placebo therapy was associated with a static diurnal profile of renin whereas quinapril clearly produced the expected reactive rise. The mean maximum rise in plasma renin activity was significantly higher (p=0.027) in the active treatment group (10.23 ± 13.16 ng A1/ml/hr) than for placebo (1.61 ± 2.54 ng A1/ml/hr).

6.3.6 Angiotensin II concentration

Angiotensin II (AngII) concentrations at baseline showed a high degree of inter-subject variability. Mean Ang II concentrations were similar in the two treatment groups; quinapril 21.53 (6.66) fmol/ml, placebo 28.84 (15.53) fmol/ml (p=0.148). As expected, mean Ang II concentration fell in the active treatment group compared to placebo (Figure 6.8). There was however no statistically significant differences in Ang II concentration between treatment groups at any time point. Mean maximum fall in Ang II was 7.40 (5.84) fmol/ml for quinapril and 8.75 (8.00) for placebo (p=N.S.).

6.4 DISCUSSION

6.4.1 Haemodynamic response

This is the first reported study in a placebo controlled setting of the haemodynamic response to the commencement of the ACE inhibitor quinapril in its recommended starting dose, 2.5 mg, in patients with stable CHF. The treatment was well tolerated with no episodes of symptomatic hypotension. Considerable inhibition of plasma ACE activity was achieved for 24 hours following this relatively small dose of quinapril.

The observed fall in mean arterial pressure was moderate and appears to be of a similar magnitude to that seen in previous open studies of quinapril in CHF. The observed mean fall of 12 mm Hg in the present study compares with 14 mm Hg seen with 5mg p.o. (Holt et al 1986), 12 mm Hg with 2.5mg p.o. (Nieminen and Kupari 1990) and
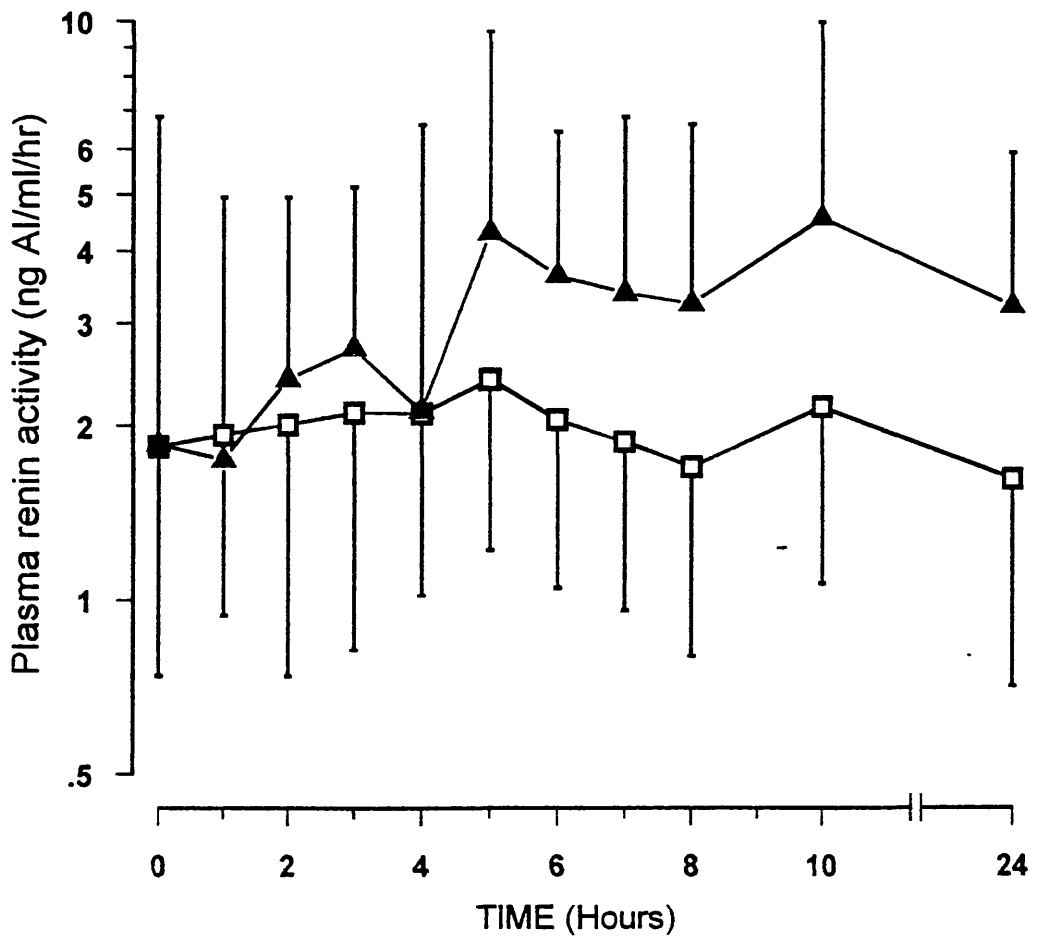
Figure 6.7:
Plasma renin activity (ng AI/ml/hr) following quinapril 2.5mg p.o. (▲)
or placebo (□) in patients with congestive heart failure.
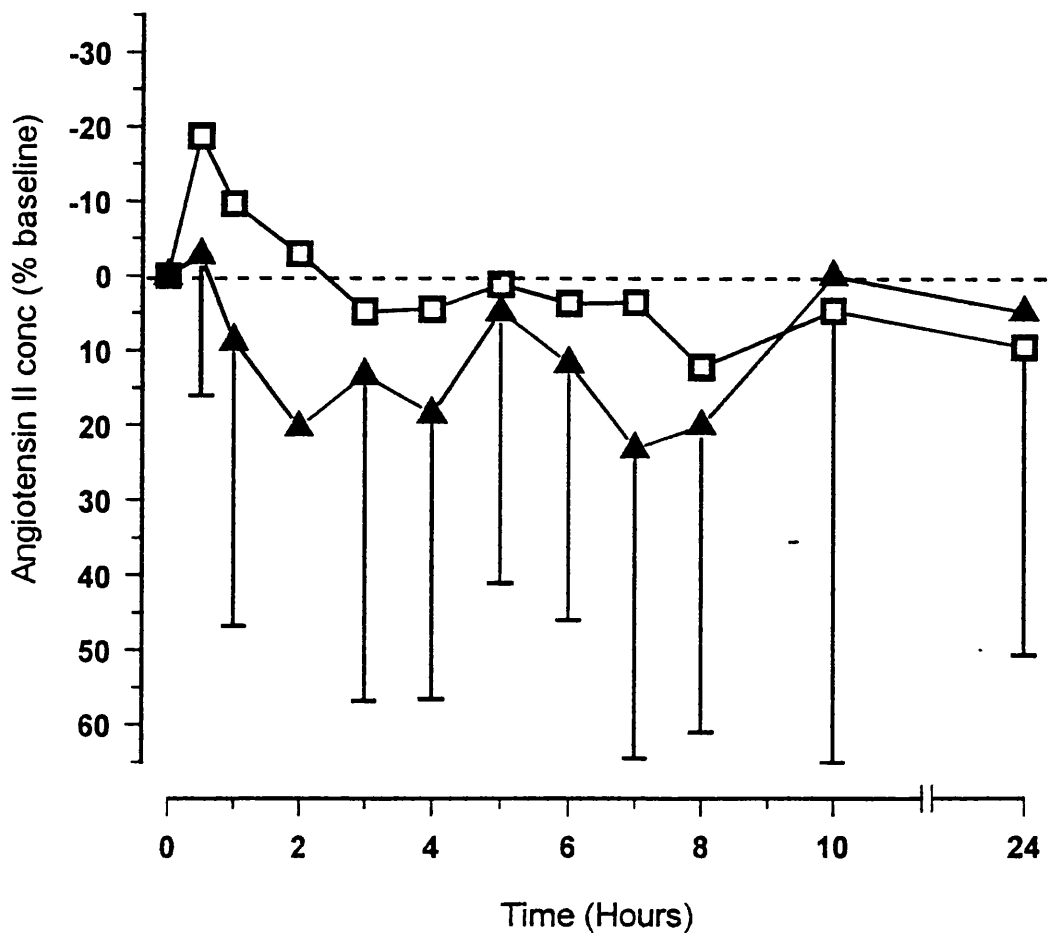
Figure 6.8:
Plasma angiotensin II concentration (% baseline) following quinapril
2.5mg p.o. (▲) or placebo (□) in patients with congestive heart failure.

12 mm Hg seen with doses between 2.5-10 mg p.o. (Sedman and Posvar 1989).

The timing of the maximum blood pressure fall seen in the present study is somewhat later than previously reported. The maximum blood pressure fall was seen at 5 hours compared with the 2-2.5 hours to maximal blood pressure response seen in previous studies of quinapril in CHF eg 5mg p.o.(Holt et al 1986), 2.5-10mg p.o. (Nieminen & Kupari 1990). In addition there would appear to be differences in the duration of effect. While the present study recorded a significant fall in blood pressure to 10 hours post dose, a previous study in heart failure described haemodynamic changes which were no longer different from baseline by 4 hours after a single dose of quinapril 5mg p.o.(Holt et al 1986). Haemodynamic monitoring was discontinued at 4 hours post dose, and that time although not statistically different, mean arterial blood pressure was 93.1±4.5 mmHg at baseline compared to 77.5±7.5 mmHg at 4 hours. Any continued effect on mean arterial pressure after this point would therefore not have been detected and the time to peak effect underestimated. Thus, while the time course of the blood-pressure lowering effect of quinapril has been compared to that of captopril with a peak effect at 1.5-3 hours (Holt et al 1986, Wadworth and Brogden 1991), the present study suggests that in heart failure a more accurate comparison may be with enalapril, with which the maximum haemodynamic changes are seen at 4-6 hours post dose (Todd & Heel 1986). If the reported greater affinity of quinapril for tissue ACE was relevant to the BP response, a relatively greater fall in BP than seen here would have been expected. Although a direct comparison between the responses to 2.5mg of either enalapril or quinapril in the studies reported in this thesis cannot be made, the observations reported do not suggest that BP response depends upon inhibition of tissue rather than plasma ACE. The time to peak effect and duration of blood pressure response appear to be greater than previously suggested.

The lack of effect of quinapril on heart rate is is in keeping with other studies of

acute ACE inhibition, the lack of reflex tachycardia in the face of a fall in blood pressure

being characteristic of these drugs. Enalapril has been shown to elicit a fall in heart rate

despite a significant fall in blood pressure in patients with heart failure (MacFadyen et al

1991) and a similar phenomenon has been observed with perindopril p.o. and perindoprilat

i.v. (Chapter 5).

6.4.2 Pharmacokinetic profile

The present study indicates that peak concentrations of quinapril occur at 2-3

hours post dose, in agreement with previous studies (Holt et al 1986). Quinaprilat levels

were not reported in the previous study. The 3-4 hours to peak quinaprilat concentration is

somewhat longer than the time to peak in normal volunteers of 2 hours (Ferry et al 1987,

Horvath et al 1990, Elliott et al 1992).

The concentration-time profiles of the parent ester quinapril and the active

metabolite quinaprilat differ from those seen with other ester prodrug ACE inhibitors in

this setting. The concentration profiles of enalapril and perindopril show relatively greater

circulating concentrations of the ester in relation to the subsequent appearance of a

protracted plateau concentration of the diacid metabolite (Chapter 5). The present study

shows comparable quantitative profiles for parent ester and diacid metabolite after a single

dose of quinapril 2.5 mg (Figure 6.6). During chronic dosing, plasma concentrations of

quinaprilat have been reported to be approximately four-fold higher than those of the

parent ester (Olson et al 1989). In keeping with the relatively large quantities of potent

diacid the profile of plasma ACE inhibition with quinapril in the present study is greater

than that seen following single doses of perindopril 2 mg p.o. and enalapril 2.5 mg p.o. in

CHF but with a lesser fall in blood pressure (Chapter 5). This may reflect inadequacy of the plasma ACE profile as an index of drug effect or may represent differential interactions of individual ACE inhibitors with plasma and tissue based renin-angiotensin systems. The significance of the atypical pharmacokinetic profile of quinapril is unclear.

Clearance of both quinapril and quinaprilat are directly related to creatinine clearance for healthy volunteers (Begg et al 1990) and for normotensive patients (Halstenson et al 1992). As with all ACE inhibitors a number of factors are likely to contribute to the differing pharmacokinetic profiles of quinapril in elderly patients with CHF. It is possible that the delay to peak drug concentration seen in the present study reflects a degree of renal impairment, as indicated by the relatively high baseline mean urea and creatinine levels. Such renal impairment may be related to age or a consequence of heart failure. Changes in drug metabolism secondary to age and as a result of CHF may be contributory.

The mean maximum plasma concentration of quinaprilat seen in the present study (51 ng/ml) is very similar to that seen in normal volunteers following a single dose of 2.5mg p.o. (Elliott et al 1992). That quinaprilat remained detectable 24 hours after a single 2.5mg dose is not surprising given its detection 72 hours after the same dose in normal volunteers (Elliot et al 1992). The concentration of drug detectable at 24 hours is higher than seen at the same stage in normal volunteers (4.4 ng/ml cf. 2ng/ml). This is likely to reflect differences in renal function and drug clearance between healthy volunteers and the older patient population of the present study who have concomitant disease.

The elimination half-life of quinaprilat has previously been estimated at 2 hours (Wadworth & Brogden 1991), a value which is perhaps inconsistent with the prolonged duration of action of quinapril. A previous study (Elliott et al 1992) demonstrated that

with a more sensitive assay than that used previously and a longer sampling period the elimination half-life approximated to 26 hours in normal subjects. The sampling period of 24 hours in the present study was too short to estimate the terminal $t_{1/2}$, but over the period 8-24 hours plasma concentrations decayed with a calculated $t_{1/2}$ of at least 6 hours. This is supporting evidence for the value of a sensitive assay in defining the plasma $t_{1/2}$. The pharmacokinetics of all long acting ACE inhibitors are such that estimates of $t_{1/2}$ are heavily dependent upon the sampling period and assay sensitivity. Estimates based on insensitive assays are of limited clinical relevance. The limit of detection of our assay is approximately 0.1 ng/ml as compared to the 5 ng/ml of the original assay (Olson et al 1989).

Although maximum quinaprilat concentrations are dose related, persisting low levels of drug during the elimination phase have been found with a number of other ACE inhibitors and are thought to reflect slow dissociation of the drug from tissue binding sites (Lees et al 1989). A formal study using a prolonged sampling period and a sensitive assay in patients with CHF would be required to establish the effect of the condition on the pharmacokinetics of quinapril, and the impact of the drug on the tissue based system derived from the extended pharmacokinetic profile.

The profile of plasma ACE inhibition in the present study is broadly comparable to that seen following a single dose of quinapril 2.5 mg p.o. in normal volunteers. Although direct comparison is not possible the degree of residual enzyme inhibition at 24 hours appears to be somewhat higher in the patients with CHF compared to the volunteers studied previously, 63% inhibition in CHF compared to 50% in volunters (Elliott et al 1992). This is again likely to reflect relatively slower drug clearance in the present population.

Although there is a poor correlation between the degree of circulating ACE inhibition and the blood pressure response following introduction of ACE inhibitor therapy, no dissociation between the two parameters was seen in this study. Quinapril has been reported as less often associated with first-dose hypotension (0.4% incidence) than either enalapril (1.5%) or captopril (2.2%) (Wadworth & Brogden 1991). These estimates do not reflect equivalent drug exposure within the general population and are unlikely to reflect the response in CHF. At present, quinapril is indicated for the treatment of CHF in doses of 10-20 mg daily in two divided doses. The present study indicates the efficacy of a single 2.5mg oral dose in inhibiting plasma ACE over 24 hours in patients with CHF.

The fall in angiotensin II following quinapril iis very much less than would have been expected. The relative lack of reduction in angiotensin II may reflect inadequacy in sample handling. However large falls were seen in a number of individual patients and is reflected in the large standard deviation around the mean fall.

Quinaprilat kinetics are non-linear and characterisation of the concentration-effect relationship is dependent upon an E_{max} model, i.e., a maximum effect model (Elliott et al 1992). The study, in normal volunteers, suggested that 2.5 mg is sufficient to provide near maximum ACE inhibition. The present study has confirmed profound ACE inhibition and a significant blood pressure fall following 2.5 mg in patients with stable CHF. However the relationship between clinical efficacy of ACE inhibitors in heart failure and haemodynamic responses such as blood pressure reduction or pharmacokinetic parameters such as plasma ACE inhibition is unclear.

Doses of quinapril as low as 0.5 mg p.o. have been shown produce up to 70% inhibition of plasma ACE, although the duration of effect is reduced with reduction of

dose. In hypertension, there appears to be a relationship between antihypertensive effect and plasma drug enalaprilat concentration (Donnelly et al 1990). No information is available on the haemodynamic response and more importantly the effect on exercise tolerance and mortality in CHF in response to chronic therapy with quinapril in a dosage of 2.5mg once daily. The issue of the dose-response relationship for different ACE inhibitors with respect to clinically relevant end points, ie morbidity, exercise capacity and mortality has not yet been fully explored. However a greater improvement in exercise capacity has been reported with quinapril 10 mg and 20 mg twice daily than with 5 mg twice daily after 12 weeks of treatment in patients with heart failure (Riegger et al 1990). Further studies would be required to identify the minimum effective dose of quinapril in heart failure.

In summary, a single dose of quinapril 2.5 mg administered orally to patients with chronic, stable CHF elicited a moderate but significant fall in MAP. The profile of blood pressure response was similar to that seen with enalapril in CHF. The pharmacokinetic profile showed relatively higher concentrations of the active diacid metabolite than is seen with other ACE inhibitors. Changes in neurohormonal parameters were as expected with inhibition of ACE which was inhibited to at least 24 hours post dose.

CHAPTER 7

COMPARATIVE IN-VIVO AND EX-VIVO RAS INHIBITION FOLLOWING ACE INHIBITION IN CHF.

7.1 INTRODUCTION

The measured level of plasma ACE inhibition does not correlate well with the observed fall in blood pressure following treatment with ACE inhibitors (Unger et al 1986, Waeber et al 1980). One possible explanation for this observation is inhibition of tissue based renin-angiotensin systems independent of plasma ACE. Alternatively, the degree of activation of the renin angiotensin system and the dependence of blood pressure on the activity of the system may vary among individuals. This is certainly a factor in patients with congestive heart failure in whom the RAS is often activated by the disease process itself and by diuretic therapy.

To date the standard method of estimating the activity of the renin angiotensin system has been the measurement of plasma ACE activity on synthetic substrates in vitro (Piquilloud et al 1970, Ryan et al 1977). However a number of workers have questioned the reliability of plasma ACE activity as an indicator of the degree of activity of the RAS. The estimate of plasma ACE activity depends to some extent on the substrate used (Nussberger et al 1989). The reappearance of increasing levels of angiotensin II during ACE inhibition occurs during acute (Morton et al 1980, Swartz et al 1979, Nussberger et al 1985) and chronic (Cirillo et al 1988, Hodsman et al 1984, Nussberger et al 1989) treatment with a number of ACE inhibitor agents. This phenomenon may have important implications during therapy with ACE inhibitors. While plasma ACE appears to be inhibited for 24 hours after a sigle dose of lisinopril, the blood pressure response

152

better profiles the reduction in circulating angiotensin II concentration; blood pressure shows a maximum fall within 6 hours of dosing and then rises steadily without regaining pretreatment levels at 24 hours (Cirillo et al 1988). A different and probably more complete inhibition of the renin angiotensin system may occur with different treatment regimens with alternative agents such as enalapril twice daily (Modina et al 1986). Nussberger et al demonstrated that while the degree of inhibition of plasma ACE produced by benazepril was apparently of greater degree and duration than that produced by enalapril, the effect on plasma angiotensin II was similar (Nussberger et al 1989). Thus it has been suggested that an *accurate* assessment of the degree of inhibition of the RAS is only given by assay of the relative concentrations of angiotensin I and II rather than in-vitro measurement of plasma ACE. Even in the face of complete inhibition of plasma ACE, angiotensin II may be formed from angiotensin I by alternative enzyme activity, e.g., chymotrypsin (Thibault & Genest 1981), cathepsin G (Tonnesen et al 1982) and tonin (Boucher et al 1972).

The pathophysiological relevance of these alternative pathways of angiotensin II generation is unclear. While the measurement of angiotensin peptides may be desirable during studies of the renin angiotensin system, there are a number of problems associated with the assay of these compounds. Plasma concentrations of active angiotensin II (angiotensin-(1-8) octapeptide) lie in the low picomolar range, requiring the use of sensitive assays for their quantification. Given the short half life, 10-12 seconds, of angiotensin II in plasma of and its in vitro half life of 2-3 minutes, there is potential for generation as well as degradation of the peptide between blood sampling and assay (Nussberger et al 1986, Nussberger 1988). In addition, a number of metabolite and precursor peptides of angiotensin II are present in plasma and are generated in vitro.

Angiotensin II (Angiotensin-(1-8) octapeptide) coexists with the (1-10) decapeptide, the (2-10) nonapeptide and the (4-8) penta-, (3-8) hexa-, and (2-8) heptapeptides. These cross-react to varying degrees in the available radioimmunoassays for angiotensin II, usually based upon antisera for the amino terminal of the octapeptide. Such antisera are of limited specificity and during ACE inhibition; falling concentrations of angiotensin II have to be assayed in the presence of concentrations of angiotensin I and metabolites which are increased up to 100 times. Previous studies from this department showed differing blood pressure responses to orally administered ester ACE inhibitors, no such differences being evident with administration of the diacid metabolites of the agents (MacFadyen et al 1991, 1993). In these studies initiation of intravenous ACE inhibitor therapy was associated with a small rise in blood pressure, which was postulated to be due to displacement of angiotensin II from ACE.

One possible explanation for the difference in blood pressure responses among orally administered agents is steric hindrance between the parent ester, a weak but highly lipophilic ACE inhibitor, and the diacid metabolite, a potent but highly polar ACE inhibitor. The presence of the parent ester perindopril in vitro can alter the degree of inhibition of plasma ACE by perindoprilat, the inhibition curve being displaced to the right (Harrigan et al 1989).

An alternative explanation for the disparate blood pressure responses seen in the face of indistinguishable profiles of plasma ACE inhibition is so-called "slow, tight binding". It has been suggested that some ACE inhibitors have the property of a time dependent decrease in K_i after incubation with ACE (Ryan et al 1986). Thus it is possible that ex-vivo measurement of plasma ACE inhibition by an agent showing "slow, tight binding" is spuriously high when the assay is carried out weeks or months after the

blood sample was drawn. Thus, if "slow, tight binding" occurs with perindopril to a greater extent than with enalapril, the degree of inhibition of plasma ACE observed with perindopril when assays are carried out may be spuriously higher than at the time of sampling. Thus the apparently equivalent plasma ACE inhibition may not be an accurate representation of inhibition of the RAS at the time of sampling.

Comparison of in-vivo ACE inhibition by measurement of the relative concentrations of angiotensin I and angiotensin II, with ex-vivo ACE inhibition by measurement of plasma ACE inhibition may reveal any temporal discrepancy between the two. In the case of "slow, tight binding" of perindoprilat as the explanation for the disparate blood pressure responses to enalapril and perindopril, a temporal dissociation between in-vivo and ex-vivo assessments of ACE activity may be expected with perindopril administered orally and perindoprilat intravenously, but not with enalapril. In the case of steric hindrance, such a temporal dissociation may be expected after perindopril p.o. but not after perindoprilat i.v.

This section describes the relative inhibition of the renin angiotensin system seen after initiation of ACE inhibitor therapy in heart failure with perindopril 2mg p.o., enalapril 2.5mg p.o., or perindoprilat 0.167mg i.v. This aspect of the study set out to compare the apparent degree of inhibition of the renin angiotensin system as assessed both by in-vitro estimation of plasma ACE inhibition and in-vivo estimation of plasma angiotensin I and II concentrations.

The aims of the study were to:

1. Compare in-vivo and ex-vivo ACE inhibition following perindopril 2mg p.o and perindoprilat 0.167mg i.v. and to compare the results with those obtained for enalapril 2.5mg p.o. The degree to which in vivo ACE inhibition is delayed or diminished compared

to ex vivo measurements for each agent will allow us to distinguish between the hypotheses of slow, tight binding and steric hindrance discussed above.

2. Establish whether angiotensin I or II concentrations rise at the time of the previously observed rise in blood pressure during infusion of perindoprilat.

7.2 PATIENTS AND METHODS

7.2.1 Patients

Forty eight elderly patients with stable, chronic congestive heart failure were recruited to the study. The analyses described here were carried out as part of the observational study described in Chapter 5. The demographic data of the study population are described in Chapter 5. The laboratory methods for the measurement of plasma ACE activity are described in Chapter 4.

7.2.2 Assay of angiotensin peptides

Angiotensin II (Ang-(1-8) octapeptide) and angiotensin I (Ang-(1-10) decapeptide) were measured using a series of specific and sensitive assays based on the methods of Nussberger et al (Nussberger et al 1985) with minor modifications (Devlin 1992). The procedure involves 3 steps: (1) extraction of angiotensin peptides by reversible adsorption to bonded phase silica; (2) separation of the angiotensin peptides by isocratic reversed phase high performance liquid chromatography (h.p.l.c.) and (3) direct linkage to a sensitive radioimmunoassay (r.i.a.).

The treatment of the patients for the purposes of blood sampling was as described in chapter 5. All patients rested supine for 45-60 minutes prior to the drawing of blood for measurement of baseline angiotensin peptide levels. Patients remained supine until 10 hours after the administration of study medication, after which they were allowed to rise

and resume normal activities. They returned to bed and again rested supine for 45-60 minutes before blood sampling for the determination of angiotensin peptide levels at 24 hours.

Procedure

Blood samples were drawn into pre-chilled glass collecting tubes on ice. The collecting tubes contained 0.5 ml inhibitor solution. The inhibitor solution contained 2% ethanol, 125 Na_2Ethylene diamine tetra-acetic acid 125 mM, o-phenanthroline 50mM, neomycine sulphate 2g/L. To prevent the in vitro generation of angiotensin II, the renin inhibitor CGP 38560, 100μM was added to the peptidase inhibitors of each tube. Samples were spun immediately at 4⁰C at 15000 r.p.m. for 15 minutes. Plasma aliquots were snap frozen and stored at -70⁰C until analysed.

Extraction

Angiotensin I and II were extracted by reversible adsorption to phenylsilyl-silica. (Bondelut-PH, Analytichem, Harbor City, California, U.S.A.). Bondelut cartridges each containing 100 mg phenylsilyl silica were placed in a vacuum manifold. Consecutive aliquots of methanol (1 ml), water (1 ml) and plasma (2 ml) were passed through the cartridge by vacuum aspiration in sequence. This procedure allows activation of the silica surface and adsorption of angiotensin peptides. Angiotensin peptides were then eluted using methanol (0.5 ml) and collected in polypropylene tubes containing albumin buffer (0.1 M Tris buffer, pH 7.5, 5 g/L bovine plasma albumin, 0.2 g/L NaN_3). The methanol was then evaporated under air in a water bath at 40⁰C.

Chromatography
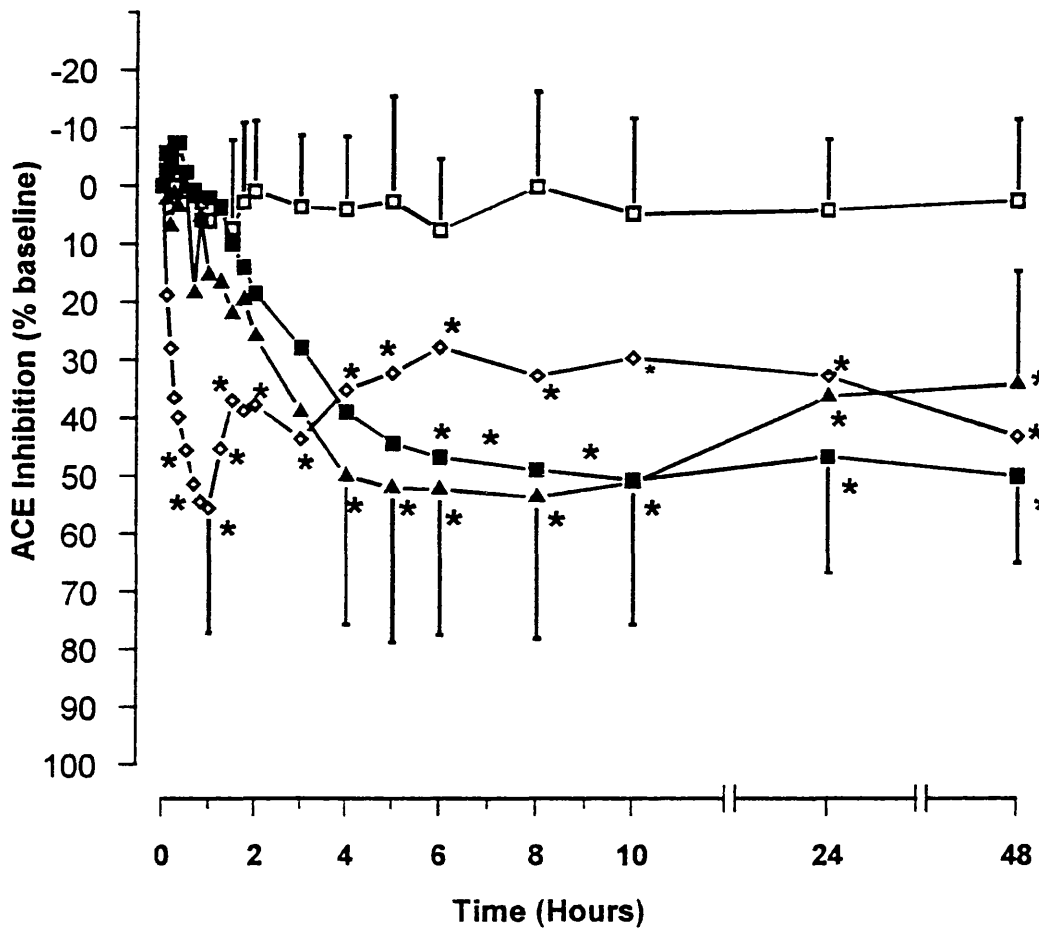
Figure 7.1:
Angiotensin converting enzyme inhibition (% baseline, mean ± 1SD)
following placebo p.o. (□), enalapril 2.5mg p.o. (▲), perindopril 2mg p.o. (■),
or perindoprilat 0.167mg i.v. (◇) in patients with congestive heart failure.

The extraction residue was redissolved 140 µL 0.1 M acetic acid. One hundred microlitres of this solution was submitted to isocratic reversed phase h.p.l.c. The system used was made up of a Hewlett Packard 1084 B liquid chromatograph with a Gilson electronic fraction collector. A 250 x 4.6 mm column containing octadecylsilyl-silica (Nucleosil C-18 5µ, Innovativ Labor, Adliswil, Switzerland) was used. The mobile phase was methanol/0.085% phosphoric acid in a ratio of 33.5 : 66.5 (Nussberger et al 1985). The flow rate was 0.5 ml / min and the column compartment was kept at a temperature of 45^0C. At the relevant retention times, previously established using 100 ng of standard angiotensin I and angiotensin II, 90 µL fractions were collected into 0.5 ml of albumin buffer.

Radioimmunoassay

Radiolabelled angiotensin II (^{125}I-ANG II, 2200 Ci / mmol, MEM Dupont) in 0.05 ml of buffer and 0.5 ml antiserum, (kindly provided by Dr. J.J.Morton, Medical Research Council Blood Pressure Unit, Western Infirmary, Glasgow) 1:26000 diluted in buffer was added to each collected chromatography fraction. Incubation was carried out for 48 hours at 4^0C. The reaction was terminated by adding 0.3 ml of dextran coated charcoal (2% suspension in water). Antibody-bound hormone was separated from free hormone by centrifugation followed by decanting (Nussberger et al 1984). A similar procedure was used for assay of angiotensin I using radiolabelled angiotensin I (^{125}I-ANG I, 2200 Ci / mmol, MEM Dupont).

Angiotensin peptide standards

Increasing amounts of of angiotensin I or II (0-20 fmol; Peninsula Labs, San Carlos, CA, U.S.A.) were added to 0.5 ml buffer and 105 µL of mobile phase. Antiserum

and radiolabelled angiotensin peptide was added as for the unknown assay samples. Standard curves were constructed relating the percentage of bound labelled peptide against unlabelled hormone. The concentration of hormone in each h.p.l.c. fraction was read from this curve.

7.3 RESULTS

7.3.1 In-vitro plasma ACE inhibition

The profiles of plasma ACE inhibition are shown in Figure 7.1. Plasma ACE activity in the placebo group remained stable throughout the study period. Analysis of variance revealed a significant main treatment effect with active therapy (p=0.009) and significant treatment-time effect (p<0.001).

The profiles of plasma ACE inhibition following enalapril 2.5mg p.o. and perindopril 2mg p.o. were similar. There were no statistical differences in the degree of plasma ACE inhibition between the two treatments at any time point. Although not statistically significantly different from one another, the absolute degree of ACE inhibition was greater for enalapril than perindopril until 10 hours post dose, after which perindopril displayed greater ACE inhibition. Maximal mean plasma ACE inhibition, as a percentage of baseline activity, was observed at 8 hours for enalapril (53.7±24.5%), and at 10 hours for perindopril (50.8±21.4%).

For the group receiving oral perindopril, plasma ACE inhibition was significantly different from placebo from 4 to 48 hours after initiation of therapy. Plasma ACE inhibition was less than in the group receiving perindoprilat i.v. between 5 minutes and 2.5 hours after initiation of therapy.

Compared to perindopril the onset of plasma ACE inhibition was faster for the group receiving enalapril and was different from placebo from 3 to 48 hours after the
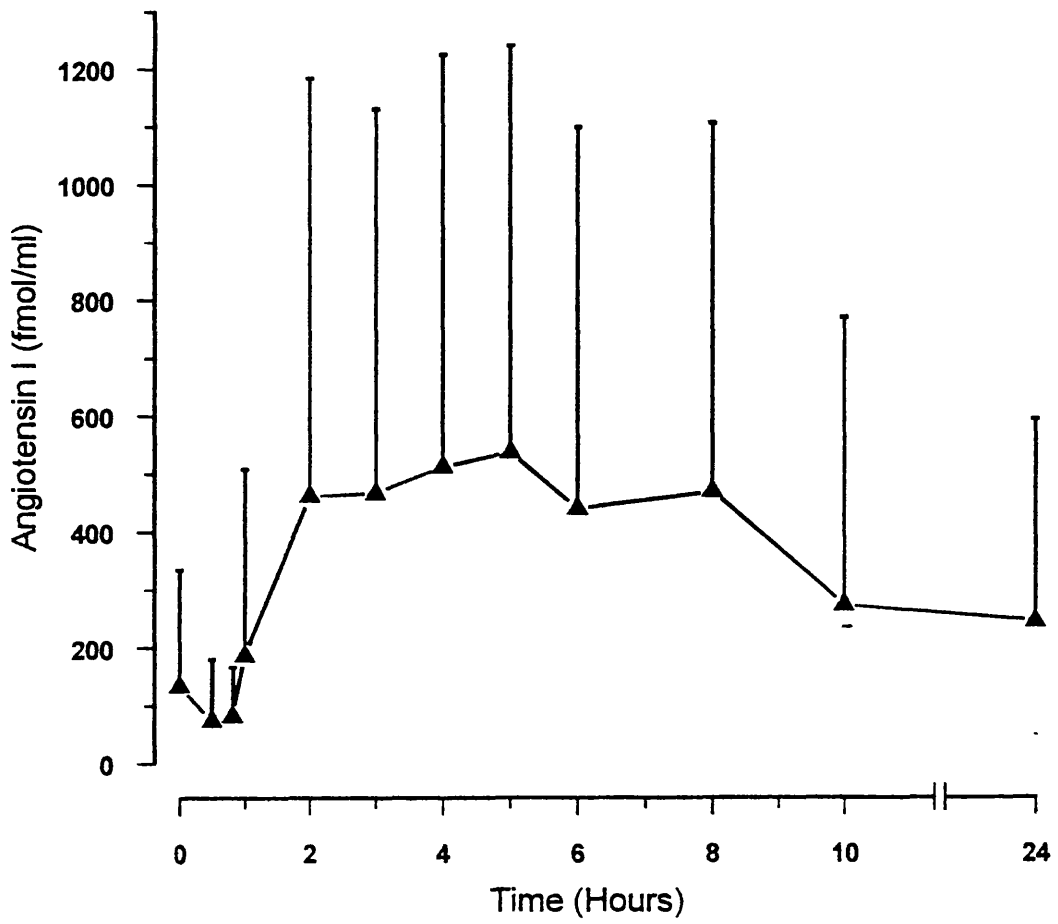
Figure 7.2:
Angiotensin I concentration (fmol/ml, mean ± 1SD) following enalapril
2.5mg p.o. (▲) in patients with congestive heart failure.
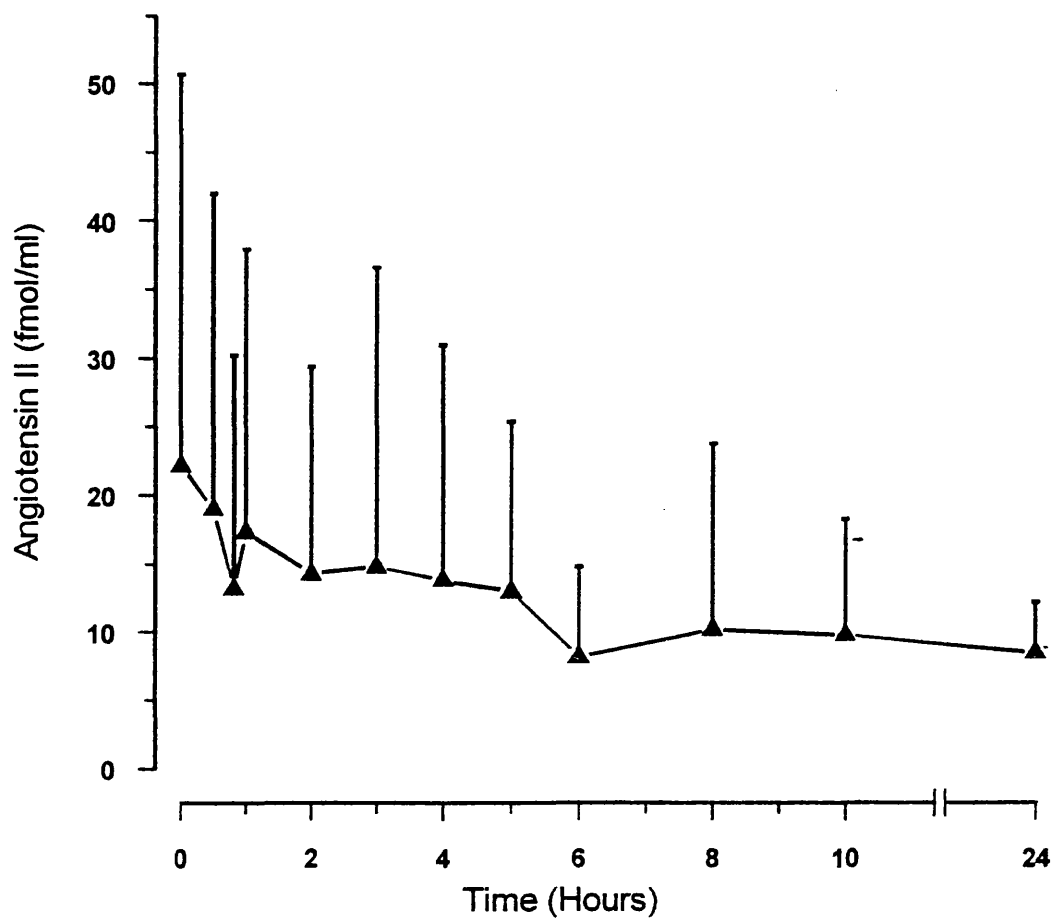
Figure 7.3:
Angiotensin II concentration (fmol/ml, mean ± 1SD) following enalapril
2.5mg p.o. (▲) in patients with congestive heart failure.

initiation of therapy. Plasma ACE inhibition was less than the group receiving

perindoprilat i.v. between 15 minutes and 2 hours after the initiation of therapy.

Plasma ACE inhibition in the group receiving perindoprilat 0.167mg i.v. was

greater than placebo from 20 minutes to 48 hours after the initiation of therapy, greater

than after perindopril p.o. from 5 minutes to 2.5 hours and greater than after enalapril

from 15 minutes to 2 hours after the start of therapy. Maximal mean ACE inhibition was

seen at the end of the 1 hour infusion period (55.6±21.6%). Following cessation of the

infusion, plasma ACE activity showed a rapid and substantial return toward baseline

levels.

Plasma ACE inhibition at 48 hours, i.e., after 2 doses of therapy, in the group

receiving enalapril was 34.1%, similar to at 24 hours after a single dose (36.3%). In the

group receiving perindopril p.o. on day 1 and day 2, plasma ACE inhibition at 48 hours

was 50% compared to 46.7% at 24 hours. In the group receiving perindoprilat i.v. on day

1 and perindopril p.o. on day 2, plasma ACE inhibition at 48 hours was 43.1% compared

to 32.8% at 24 hours.

In summary, after the first dose of study medication, i.v. perindoprilat 0.167mg

produced rapid, profound inhibition of plasma ACE which was significantly different from

placebo from 20 minutes-24 hours post dose. In addition the degree of plasma ACE

inhibition produced by this treatment was greater than that produced by perindopril 2mg

p.o. between 5 minutes and 3 hours post dose. All three active treatments produced

significant inhibition of plasma ACE between 3 and 24 hours post dose. Plasma ACE

activity was similar in all three treatment groups from 4-24 hours post dose. The degree

of inhibition of plasma ACE inhibition increased after 2 consecutive doses of perindopril

2mg p.o. and also after perindoprilat 0.167mg i.v. on day 1 followed by perindopril 2mg
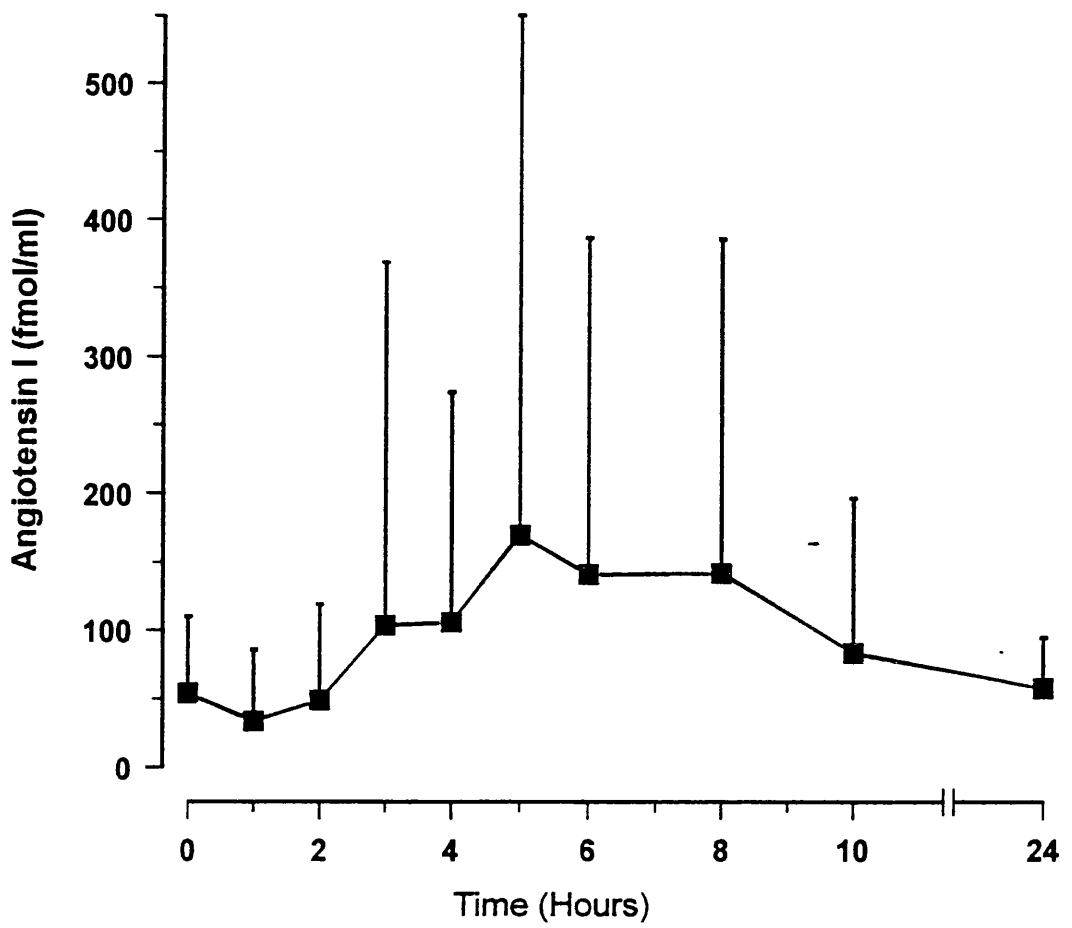
Figure 7.4:
Angiotensin I concentration (fmol/ml, mean ± 1SD) following
perindopril 2mg p.o. (■) in patients with congestive heart failure.
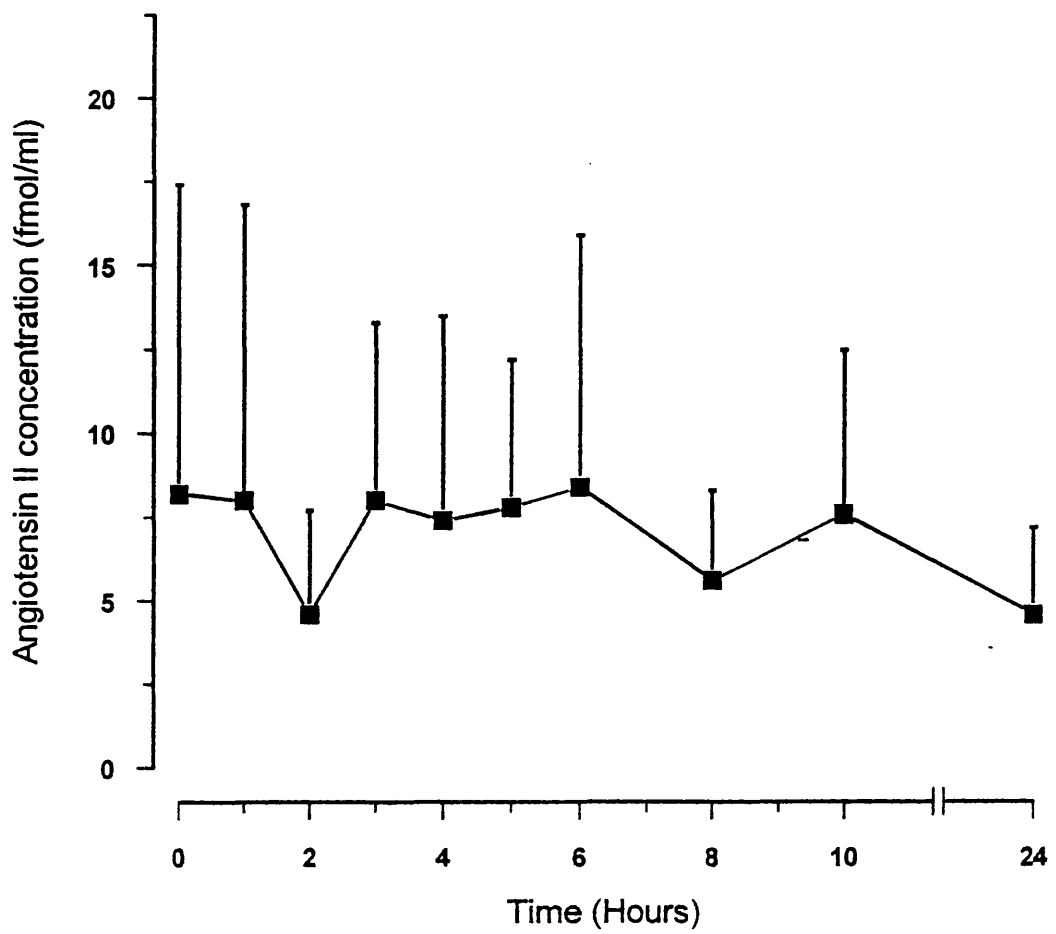
Figure 7.5:
Angiotensin II concentration (fmol/ml, mean ± 1SD) following
perindopril 2mg p.o. (■) in patients with congestive heart failure.

p.o on day 2. Although the relative increases between 24 and 48 hours were small, no such increase was seen with consecutive doses of enalapril 2.5mg p.o.

7.3.2 In-vivo ACE inhibition

Baseline

The assay for angiotensin I was hindered by relative lack of specificity of the antibody to Ang I; this resulted in a constant low level background reading of Ang I. This phenomenon was problematic where Ang I levels showed no or little rise after ACE inhibition, rendering interpretation of Ang II/Ang I+Ang II ratios difficult. Results showed wide variation among patients in terms of both baseline Ang I and Ang II levels and the response to initiation of ACE inhibitor therapy. Baseline Ang I levels (p=0.365) and Ang II (p=0.541) levels were similar among active treatment groups.

The profiles of Ang II/Ang I+Ang II ratio, as a percentage of baseline activity for enalapril 2.5mg p.o. and perindopril 2mg p.o. showed an early rise not seen following perindoprilat 0.167mg i.v. In neither case did the rise reach statistical significance.

Placebo p.o.

The profiles of Ang II/Ang I+Ang II ratio, as a percentage of baseline activity, showed an early rise with placebo therapy, suggesting increased RAS activity at this time. The ratio returned to baseline level by the 2 hour time point. There was no significant difference in the ratio of Ang II/Ang I + Ang II from baseline at any time point after 2 hours in the placebo group. Correction for the response to placebo was made for each active therapy.

Enalapril 2.5mg p.o.

Mean baseline Angiotensin I concentration was 133 (212) fmol/ml. Mean baseline
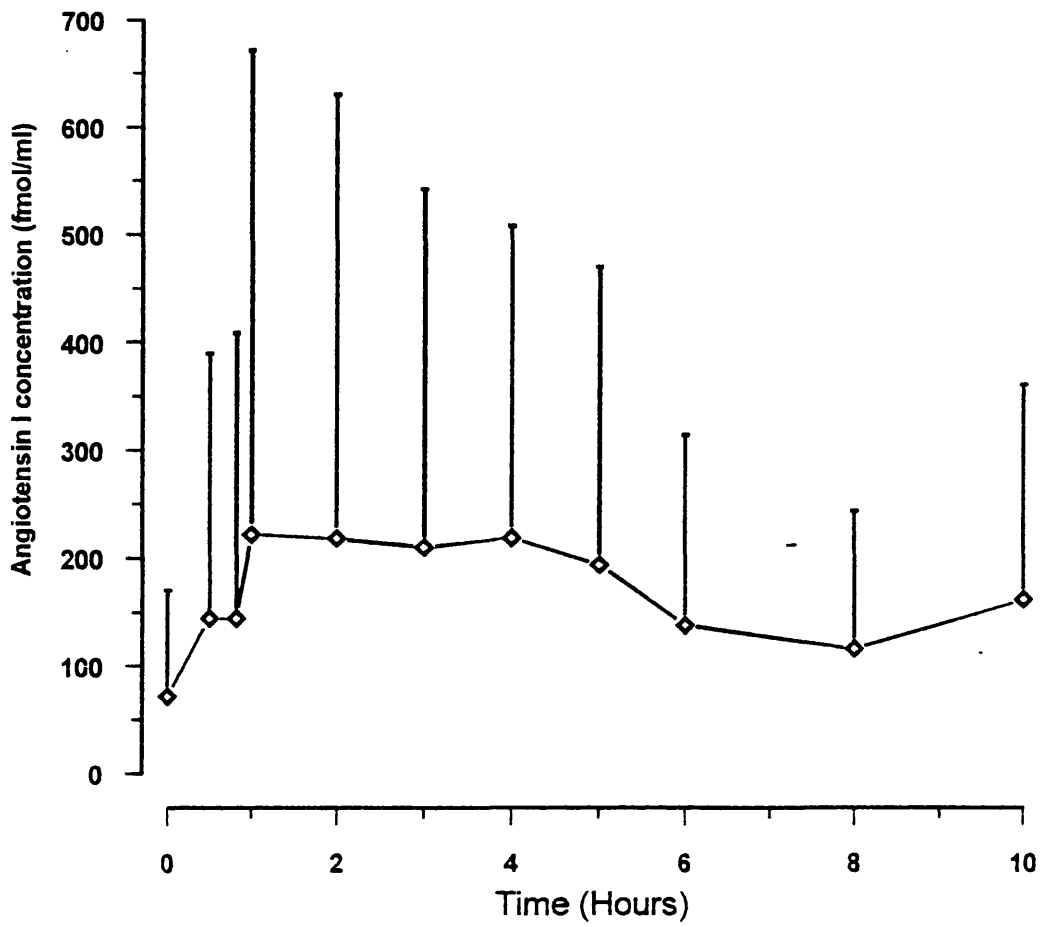
Figure 7.6:
Angiotensin I concentration (fmol/ml, mean ± 1SD) following perindoprilat
0.167mg iv in patients with congestive heart failure.
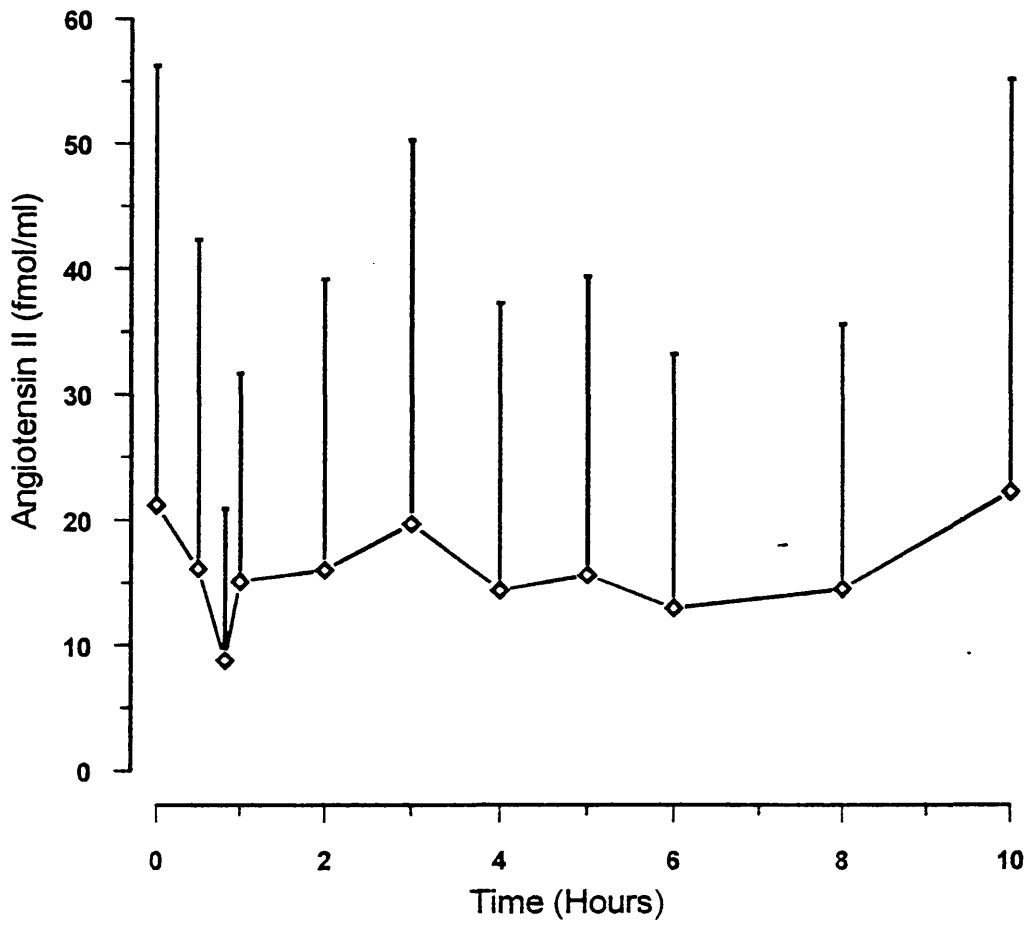
Figure 7.7:
Angiotensin II concentration (fmol/ml, mean ± 1SD) following
perindoprilat 0.167mg i.v (◊) in patients with congestive heart failure.

Angiotensin II concentration (1 S.D.) was 22 (30) fmol/ml. Mean plasma angiotensin I concentration showed an early rise and there was a plateau of increased angiotensin I concentration between 2 and 8 hours post dose. Maximum concentration (404% of baseline) was seen at 5 hours post dose (Figure 7.2). Mean angiotensin II concentration fell to 37 (80)% of baseline at 6 hours post dose (Figure 7.3).

Perindopril 2mg p.o.

Mean baseline Angiotensin I concentration was 54 (59) fmol/ml. Mean baseline Angiotensin II concentration (1 S.D.) was 11 (12) fmol/ml. Mean plasma angiotensin I concentration showed a delayed rise in comparison to that seen following enalapril. In addition the relative rise in angiotensin I concentration in relation to baseline was less than the rise seen after enalapril. The maximum increase (322% of baseline) was seen at 5 hours post dose (Figure 7.4). Mean plasma angiotensin II concentration showed an early fall to 56 (67)% of baseline at 2 hours and a later fall at 8 hours post dose but there was no discernible overall pattern to the change in angiotensin II levels (Figure 7.5).

Perindoprilat 0.167mg p.o.

Mean baseline Angiotensin I concentration was 72 (102) fmol/ml. Mean baseline Angiotensin II concentration (1 S.D.) was 21 (30) fmol/ml. Mean plasma angiotensin I concentration showed an early rise to reach a maximum (308% of baseline) at 1 hour, i.e., the end of the infusion period (Figure 7.6). Mean plasma angiotensin II concentration fell to 42 (138)% of baseline at 0.82 hours after the start of the infusion (Figure 7.7).

7.3.3 Comparative in-vivo/ ex-vivo ACE inhibition

Enalapril 2.5 mg p.o.
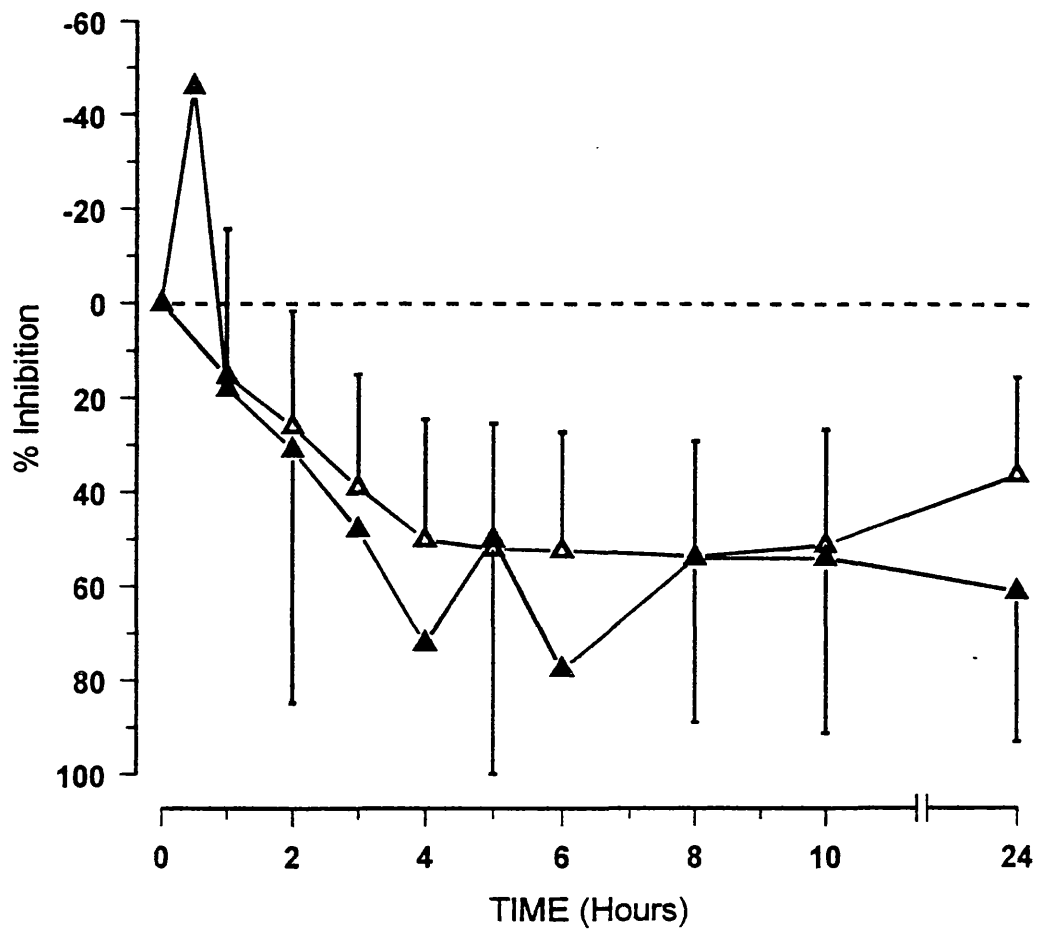
Figure 7.8:
Placebo corrected renin angiotensin system inhibition (% baseline activity) as assessed by plasma ACE inhibition (▲) and Ang II/Ang I+Ang II ratio following enalapril 2.5mg p.o. in patients with congestive heart failure.
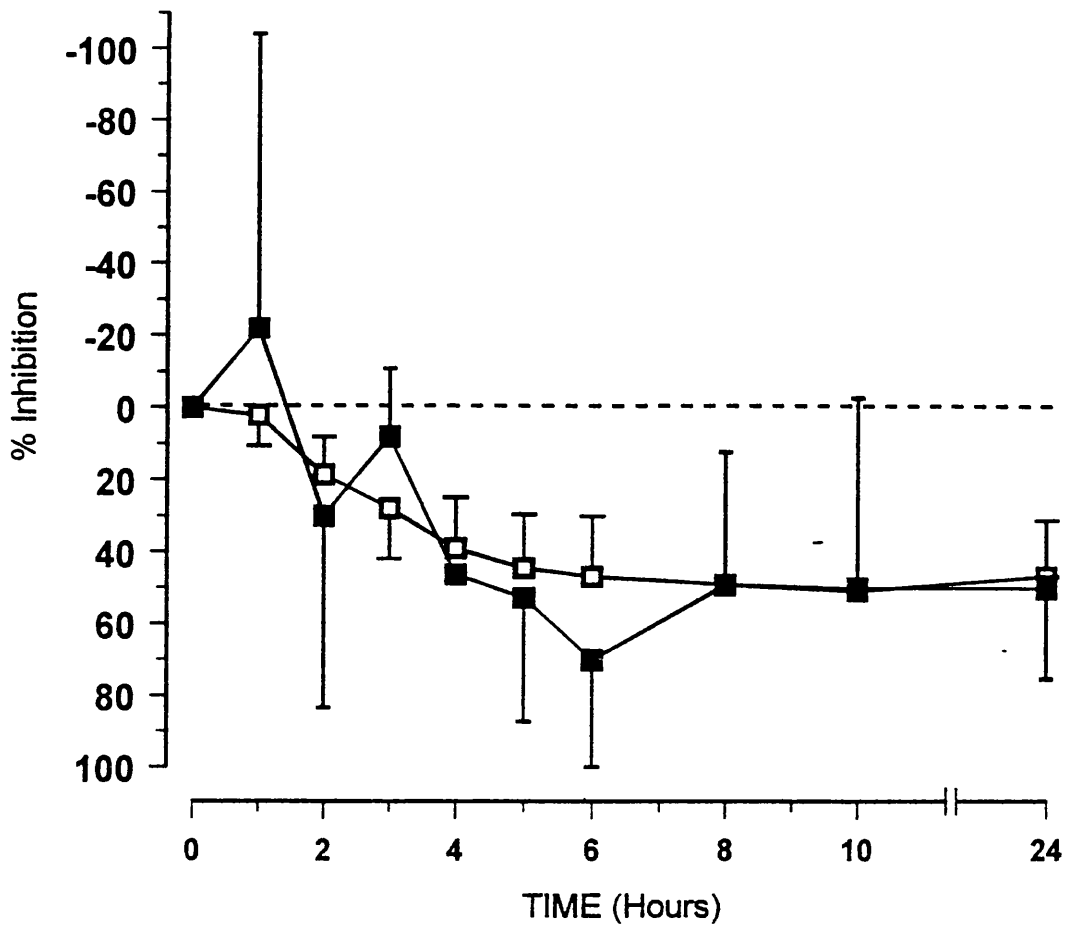
Figure 7.9:
Placebo corrected renin angiotensin system inhibition (% baseline activity) as assessed by plasma ACE inhibition (□) and Ang II/AngI+Ang II ratio (■) following perindopril 2mg p.o in patients with congestive heart failure.
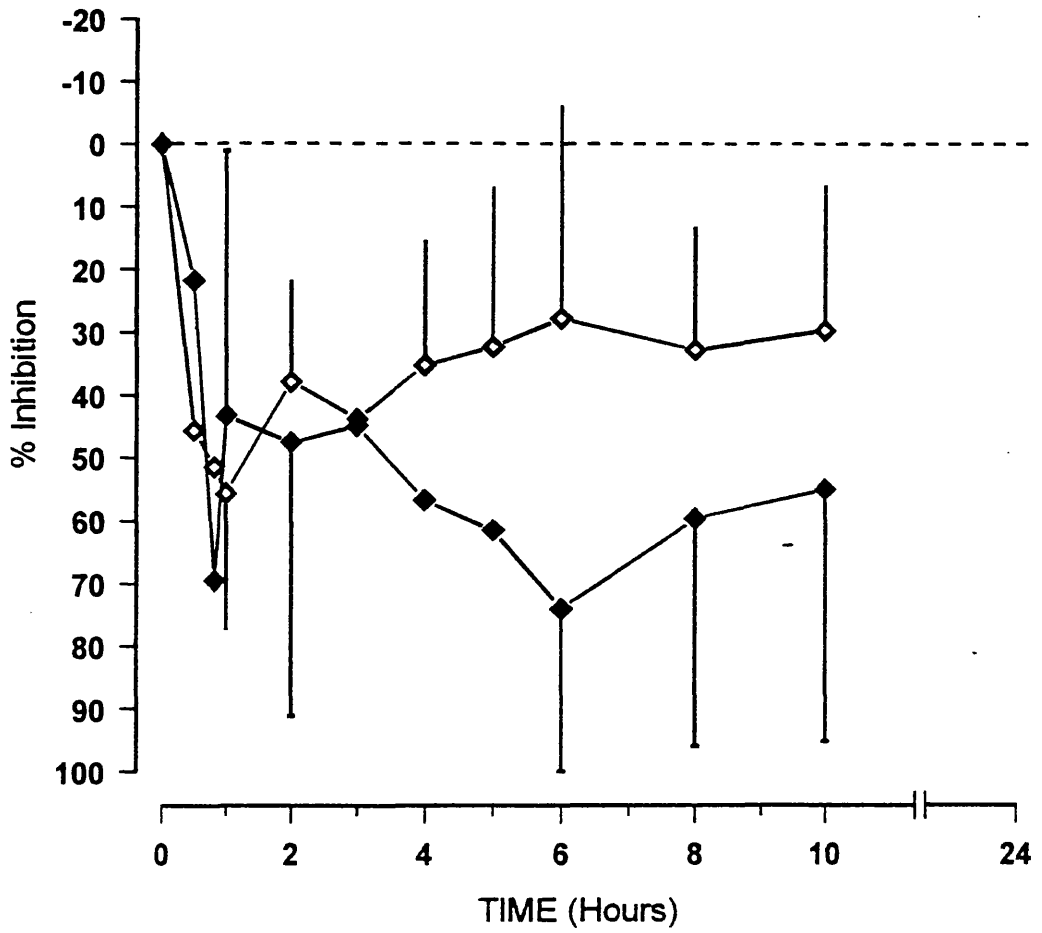
Figure 7.10:
Placebo corrected renin angiotensin system inhibition (% baseline activity)
as assessed by plasma ACE inhibition (◇) and ANg II/Ang I+Ang II ratio (◆)
following perindoprilat 0.167mg i.v. in patients with congestive heart failure.

Maximum mean ACE inhibition as indicated by minimum Ang II/Ang I+Ang II (86.9%

inhibition) ratio occurred at 5 hours post dose. Mean maximum ACE inhibition as assessed

by inhibition of plasma ACE occurred at 8.5 (5.4) hours and as assessed by AngII/Ang I +

Ang II ratio at 8.8 (8.1) hours (p=0.918) (Figure 7.8).

Perindopril 2mg p.o.

Maximum mean ACE inhibition as indicated by minimum Ang II/Ang I+Ang II

(45.0% inhibition) ratio occurred at 6 hours. Mean maximum ACE inhibition as assessed

by inhibition of plasma ACE occurred at 9.3 (5.0) hours and as assessed by Ang II/Ang

I+Ang II ratio at 6.2 (2.4) hours (p=0.128) (Figure 7.9).

Perindoprilat 0.167mg p.o.

Maximum mean ACE inhibition as indicated by minimum Ang II/Ang I+Ang II

(81.4% inhibition) ratio occurred at 0.67 hours. Mean maximum ACE inhibition as

assessed by inhibition of plasma ACE occurred at 0.9 (0.2) hours and as assessed by Ang

II/Ang I+Ang II ratio at 1.8 (2.4) hours (p=0.178) (Figure 7.10). The patterns of in-vivo

and in-vitro ACE inhibition were similar, with the nadir in the curve at the end of the 1

hour infusion period being followed by a partial return toward baseline.

7.3.4 Plasma renin activity

Plasma renin activity (PRA) showed wide variation among patients. Mean PRA

was similar among treatment groups. Mean baseline values (1 SD) were: placebo 8.59

(10.54) ng AI/ml/hr; enalapril 10.42 (10.0) ng AI/ml/hr; perindopril 5.89 (9.19) ng AI/

ml/hr ; perindoprilat 6.84 (8.02) ng AI/ml/hr (ANOVA p=0.729).
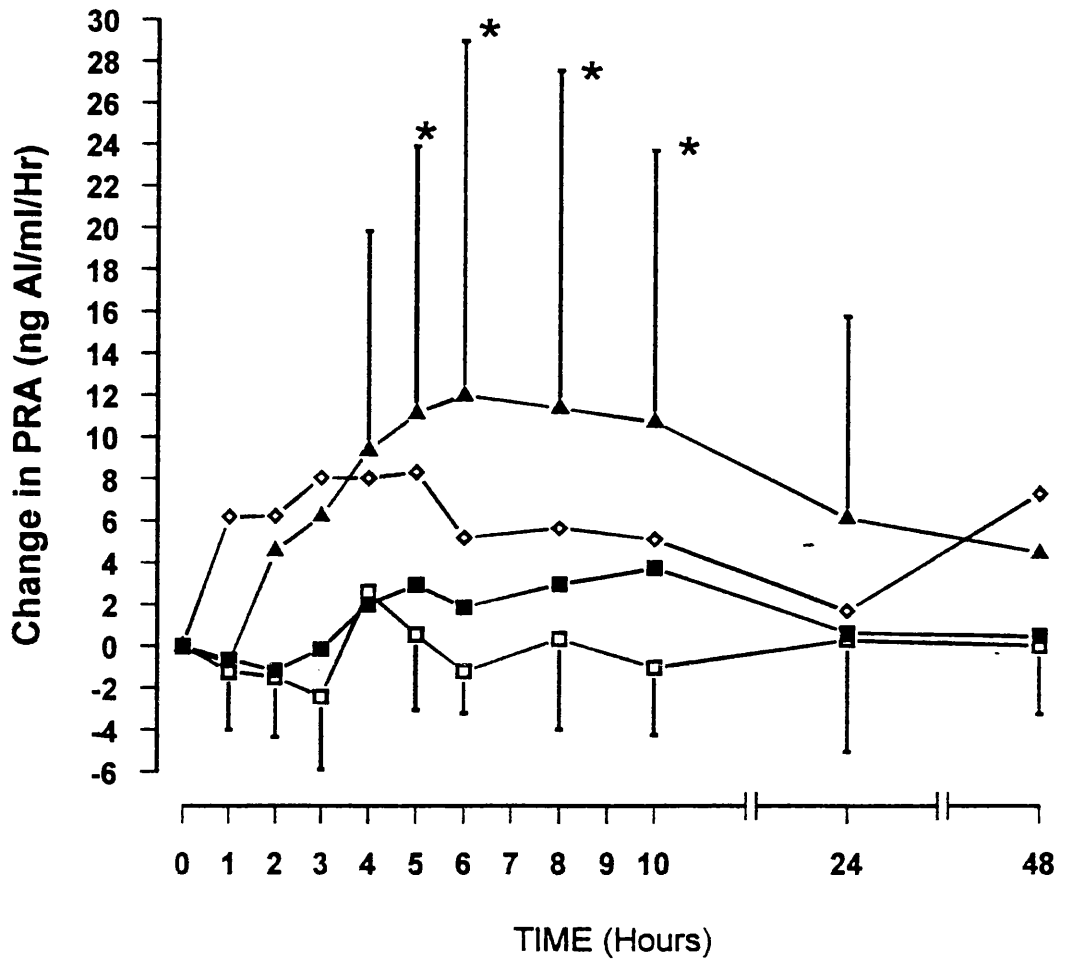
Figure 7.11:
Change in plasma renin activity (ng AI/ml/hr) following placebo p.o. +
placebo p.o.(□), enalapril 2.5mg p.o. + enalapril 2.5mg p.o.(▲),
perindopril 2mg p.o. + perindopril 2mg p.o. (■), or perindoprilat 0.167mg i.v.
+ perindopril 2mg p.o. in patients with congestive heart failure.
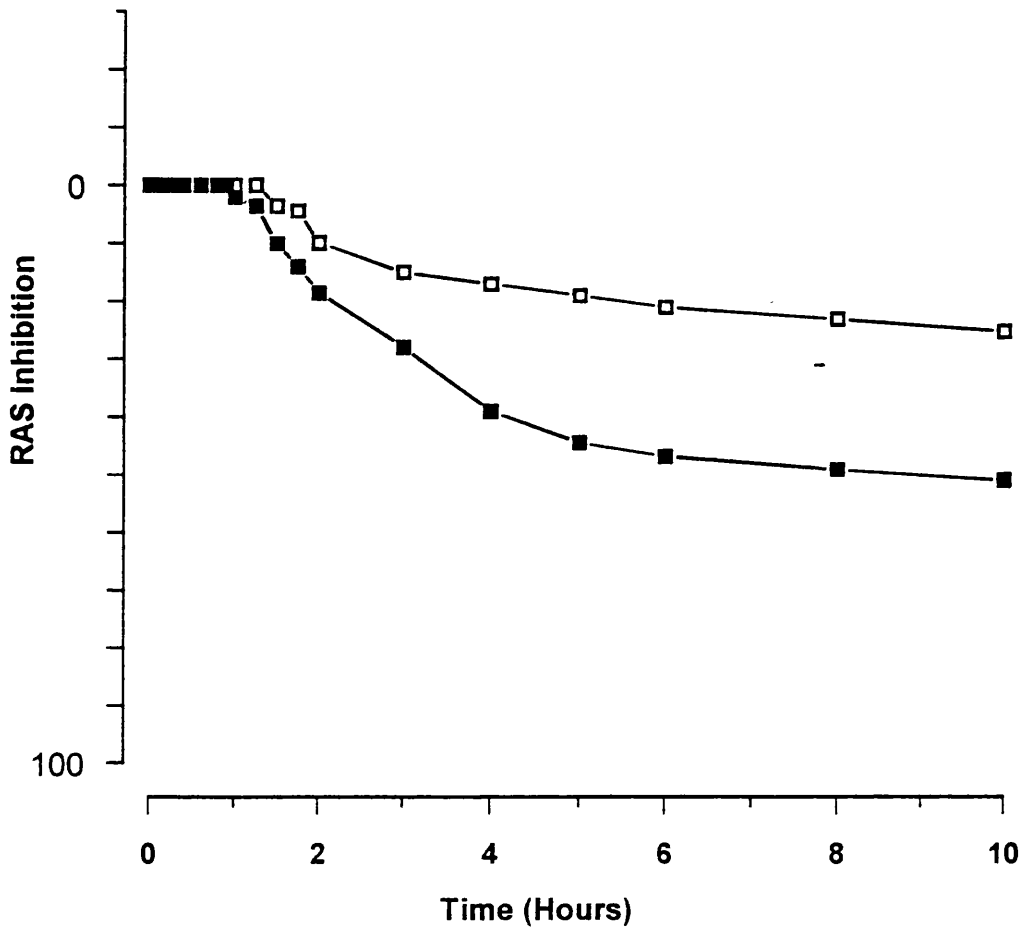
Figure 7.12:
Theoretical profile of RAS inhibition if steric hindrance were to occur.
RAS inhibition with oral ACE inhibitor therapy (□) is significantly reduced
compared to that seen with intravenous therapy (■) at any given time point.
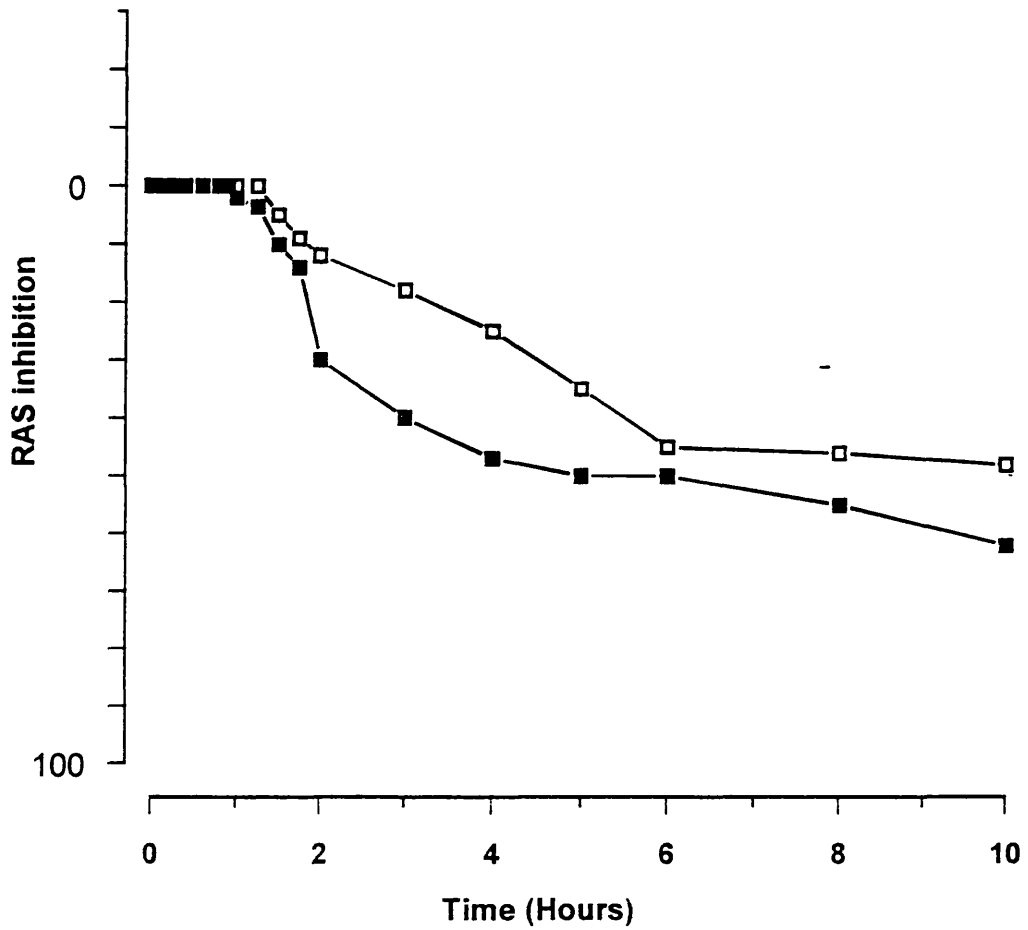
Figure 7.13:
Theoretical profile of RAS inhibition if "slow, tight binding" were to occur.
There is temporal dissociation between RAS inhibition as measured from plasma
ACE inhibition (□) compared to that measured from consideration of
angiotensin peptide concentrations (■).

There were differences among treatment groups in terms of the mean maximum rise in PRA in response to treatment. Mean maximum rise in PRA (1 SD) was: placebo 6.76 (9.24) ng AI /ml/hr; enalapril 15.64 (17.39) ng AI/ml/hr; perindopril 5.71 (8.69) ng AI/ml/hr; perindoprilat 11.06 (14.56) ng AI/ml/hr. The change in PRA was significantly greater following enalapril compared to placebo between 5-10 hours post dose (ANOVA $p<0.05$). The change in PRA was significantly greater following enalapril compared to perindopril at 6 hours (ANOVA $p<0.05$) (Figure 7.11).

7.4 DISCUSSION

In-vitro ACE inhibition as assessed by plasma ACE activity was similar following perindopril 2mg p.o. and enalapril 2.5mg p.o. The absolute values for ACE inhibition were greater following enalapril 2.5mg p.o. In addition the angiotensin I response following enalapril 2.5 mg p.o. was of earlier onset, of greater magnitude and more sustained than that following perindopril 2 mg p.o. No temporal discrepancy was demonstrated for any active therapy between in-vitro and in-vivo ACE activity. The relative degrees of in-vitro ACE inhibition following enalapril and perindopril are paralleled by the relative degrees of in-vivo ACE inhibition, both being relatively greater for enalapril. However, whereas the *absolute* values for in-vitro ACE inhibition are similar between the two active oral treatments, in-vivo ACE inhibition is relatively greater following enalapril 2.5mg p.o.

No rise in blood pressure seen after commencement of perindoprilat i.v. A trend to an increase in blood pressure was seen soon after both oral enalapril and perindopril. This rise in blood pressure was paralleled by a rise in angiotensin I, a fall in angiotensin II and a fall in AII/AI+AII ratio over the same time period with both oral treatments. However neither the rise in blood pressure nor the rise in AII/AI+AII ratio was statistically

significant for either treatment. Only a relatively slight rise in AII/AI+AII ratio was observed during or after perindoprilat i.v. infusion but with no concomitant rise in blood pressure. The lack of blood pressure rise with intravenous therapy contradicts the findings of a previous study (MacFadyen et al 1993).

One theory for the differential blood pressure response to oral perindopril and intravenous perindoprilat as compared to placebo was steric hindrance between parent ester perindopril and active diacid perindoprilat. This study presents no evidence of steric hindrance between perindopril and perindoprilat in a clinical setting. The correlation between inhibition of plasma ACE and AII/AI+AII ratio, with no temporal discrepancy, does not support the theory and initially suggests that measurement of plasma ACE activity represents an accurate assessment of the activity of the circulating RAS. The study also fails to support the theory that perindopril shows slow, tight binding to ACE. The theoretical profiles of RAS inhibition in the cases of steric hindrance and slow tight binding are show in Figures 7.12 and 7.13 respectively.

Although plasma ACE inhibition was more prolonged after perindopril 2mg p.o. than after enalapril 2.5mg p.o., and the trend of plasma ACE inhibition was downward at 24 hours, no greater inhibition of plasma ACE was seen at 48 hours than at 24 hours.

The differing plasma renin activity responses to the active therapies are of note. The rise in PRA seen after enalapril 2.5mg p.o. was significantly greater than after placebo between 4 and 10 hours post dose. No difference from placebo was seen with either perindopril 2mg p.o. or perindoprilat 0.167mg i.v. However the rise after perindoprilat i.v. was quantitatively greater than with oral perindopril. Qualitatively the pattern of PRA response differs between perindopril p.o. and perindoprilat i.v., the rise being earlier, and sustained with perindoprilat i.v. While the PRA profile after enalapril may reflect the fall in

blood pressure seen after this treatment, it may alternatively reflect a greater inhibition of the RAS than is seen following perindopril p.o. or perindoprilat i.v. in spite of the apparently similar plasma ACE inhibition seen with all three treatments. This hypothesis is supported by the greater inhibition of the RAS by enalapril compared to perindopril and perindoprilat suggested by AII/AI+AII ratios.

Measurement of angiotensin peptides rather than plasma ACE has been suggested to be necessary for true estimation of the state of activity of the RAS (Nussberger et al 1989). The measurement of angiotensin peptides theoretically allows in-vivo investigation of ACE activity whereas plasma ACE may overestimate the degree of enzyme inhibition. The current assay for angiotensin peptides proved difficult to apply to a large number of patients in a clinical setting. the levels of Ang peptides obtained were rather higher than those reported from other groups with expertise in this field (Nussberger et al 1986, 1988). However these elevated values may indicate activation of the RAS in the current patient population.

CHAPTER 8

DETERMINANTS OF THE BLOOD PRESSURE RESPONSE TO THE FIRST DOSE OF ACE INHIBITOR IN MILD TO MODERATE CHF.

8.1 INTRODUCTION

In spite of clear evidence of their safety, tolerability and efficacy in all grades of chronic CHF (The CONSENSUS Trial Study Group 1987, Pfeffer et al 1992, The SOLVD investigators 1991, 1992) and in patients with heart failure following acute myocardial infarction (AIRE Study Group 1993, Kober et al 1995), a significant proportion of patients who may benefit from treatment are not prescribed an ACE inhibitor (Rajfer 1983, Clarke et al 1994) and rates of use vary widely (Philbin et al 1996). Concerns regarding first-dose hypotension relate to the risk of organ (renal, myocardial or cerebral) hypoperfusion which may result.

The overall incidence of first-dose hypotension remains unclear; however the occurrence of first dose hypotension was of sufficient magnitude to warrant a reduction in the starting dose of enalapril in one of the early trials of ACE inhibitors in CHF (The CONSENSUS Trial Study Group 1987). A number of case reports (Cleland et al 1985), and the well-published CONSENSUS II study have highlighted the phenomenon. Moreover in the CONSENSUS II study, mortality was higher among those patients who developed first-dose hypotension after initiation of therapy with enalapril (Swedberg et al 1992). On the basis of these reports a variety of anecdotal protocols have been developed for the initiation of ACE inhibitor treatment in CHF (McMurray et al 1989). Many of these have involved admission to hospital and variable periods of diuretic withdrawal and blood pressure observation. The possibility of first-dose hypotension is recognised within the data sheets of all ACE inhibitors currently marketed in the U.K. On the basis of the

possibility of a fall in blood pressure on initiation of therapy, the British National

Formulary continues to advise both admission to hospital, and temporary diuretic

withdrawal for certain "high-risk" categories of patient with CHF (British National

Formulary September 1996). Such procedures have obvious implications for both patient

and physician in terms of inconvenience and allocation of resources and may contribute to

the failure to prescribe therapy appropriately.

A number of physiological and biochemical parameters may help to identify

patients at high risk of significant first-dose hypotension. Hyponatraemia and hypo-

volaemia associated with high doses of loop diuretic are risk factors in patients with more

severe CHF (Cleland et al 1985, Hodsman et al 1983, Packer et al 1986). However as the

patient population for whom ACE inhibition is appropriate therapy has widened, patients

with these easily identifiable, high risk markers have become the minority of those being

commenced on therapy. Hyponatraemia and hypovolaemia are seldom found in those with

mild to moderate CHF, for whom the indicators of the likelihood of a significant fall in

blood pressure have rarely been investigated.

To date only a single study has investigated in a systematic manner the

determinants of the blood pressure response to ACE inhibition in mild to moderate CHF

(Motwani et al 1994). These authors used stepwise regression analysis to study the

possible determinants of the response to a single oral dose of captopril 25mg in 36 patients

with mild to moderate CHF. Their results suggested that over 70 % of the variability in

mean arterial pressure response could be explained on the basis of 3 factors. These were:

the decrease in serum angiotensin II after initiation of treatment (F ratio =10.3, $p<0.01$);

the decrease in serum noradrenaline after initiation of treatment (F ratio = 8, $p=0.02$); and

the pre-treatment mean arterial blood pressure (F ratio = 5.6, $p=0.04$). It is of note that of

Table 8.1

Design of studies 1-3

Study 1   72 patients, n=12   captopril 6.25mg p.o./ enalapril 2.5mg p.o. /
                                perindopril 2mg p.o. / enalaprilat 0.25 mg/hr iv
                                (6 hours) / perindoprilat 0.167mg/hr iv (6 hours) /placebo

Study 2   48 patients, n=12   enalapril 2.5mg p.o. / perindopril 2mg p.o. /
                                perindoprilat 0.167 mg/hr iv (1 hour) / placebo

Study 3   24 patients, n=12   quinapril 2.5mg p.o./ placebo

the parameters contained within the model only one, baseline MAP, is a baseline parameter, and that this had the weakest association with the blood pressure response.

The analysis described here examines the first-dose blood pressure response in patients treated with one of a number of ACE inhibitor formulations or with placebo. The aims of the study were firstly, to test whether the variability in pharmacodynamic response to acute ACE inhibition, as assessed by the change in systemic blood pressure, was determined by pharmacokinetic factors, and if inter-agent differences could be identified in this regard. Secondly, to investigate the association between the blood pressure response and a variety of physiological variables in an attempt to quantify the magnitude of the relationship between routinely available clinical and laboratory parameters and the fall in blood pressure in response to a single dose of ACE inhibitor.

8.2 METHODS

8.2.1 Patients

A database was constructed from a number of studies of the haemodynamic response to the first dose of ACE inhibitor carried out within the University of Glasgow Department of Medicine & Therapeutics (MacFadyen et al 1991, MacFadyen et al 1993, Squire et al 1994, Squire et al 1996). The database consisted of 144 patients (51-88 years, 99 men) with CHF, each of whom was recruited to one of the above double-blind, placebo-controlled, parallel group studies. The design of each study is shown in Table 8.1. Inclusion and exclusion criteria were identical for each study and are detailed in Chapter 4. All studies were approved by the local ethical review committee and each patient gave written informed consent to participation. All had stable, chronic CHF, New York Heart Association functional class II-IV, and were symptomatic on stable doses of diuretic.

None had previously been exposed to ACE inhibitor therapy. The procedures for commencement of therapy, haemodynamic monitoring and blood sampling were identical in each study and are described fully in Chapters 4 and 5.

8.2.2 Haemodynamic observations

The procedure for haemodynamic observation and blood sampling was as described in Chapter 4. For each of the 3 studies the mean placebo response was estimated by smoothing and averaging the individual baseline corrected placebo blood pressure measurements. In the placebo group missing blood pressure measurements were estimated by taking the mean of the individual preceding and subsequent measurements. Outliers were defined as values < 70 % or > 130 % of the nearer of the preceding or subsequent value and were replaced by the mean of the preceding and subsequent measurements. Three point averaging was applied to smooth each individual blood pressure profile and an average placebo profile constructed for each individual study. For each subject a baseline correction was made to mean arterial pressure at each time point by subtracting the baseline mean arterial pressure. Baseline corrected mean arterial pressure values in the active treatment groups were further corrected by subtracting the appropriate baseline corrected average placebo response.

8.2.3 Pharmacokinetic analysis

Patients were randomised to receive one of the following treatments: oral perindopril 2mg (n=24); oral enalapril 2.5mg (n=24); oral quinapril 2.5mg (n=12); intravenous perindoprilat 0.167mg/hour for 6 hours (n=12); intravenous perindoprilat 0.167mg/hour for 1 hour (n=12); intravenous enalaprilat 0.25mg/hour for 6 hours (n=12). Within each study a control group (n=12) was administered oral and/or intravenous

placebo as appropriate (Table 8.1). In the setting of a double-blind study, plasma concentrations of captopril cannot be determined due to the requirement for special preparation of plasma. For this reason the 12 patients who received captopril were not included in the present analysis. The pharmacodynamic (PD) effect of each of the orally administered ACE inhibitors used in these studies is mediated by the active diacid metabolite of the parent ester drug. While it is possible to model the absorption of orally administered ACE inhibitor and its conversion to the active diacid metabolite, only the formation, disposition and elimination of each active diacid metabolite was modelled. The pharmacokinetic (PK) analysis thus included a total of 132 patients: 48 who received perindoprilat (24 perindopril p.o., 24 perindoprilat i.v.), 36 patients who received enalaprilat (24 enalapril p.o., 12 enalaprilat i.v.) and 12 who received quinaprilat (quinapril p.o.). The total number of placebo treated patients was 36. When carrying out PK modelling, molar doses and concentration levels were used, with 1 mole of the parent ester ACE inhibitor generating 1 mole of the active metabolite.

The pharmacokinetics of each drug were evaluated using a number of conventional compartmental models. The fit of each model to the data was assessed by the residual sum of squares (RSS), the Akaike Information Criterion (AIC)(Yamaoka et al 1978) and the Schwarz Information Criterion (SIC). Model fitting was carried out using the computer package STATIS (Clydesoft, UK).

8.2.4 Pharmacodynamic modelling

For each individual the area under the baseline and placebo corrected MAP curve up to 10 hours after initiation of therapy (MAP10) was calculated using a linear trapezoidal method. This estimate was used as a direct measure of the magnitude of the

Table 8.2
Summary of baseline clinical and laboratory variables.

Patient characteristics

	Age (yrs)	NYHA class (II/III/IV)	MAP (mm Hg)	PRA (ng AI/ml/hr)	Sodium (mM)	Furosemide (mg/day)	Aetiology of CHF (IHD/HT/AC/V/D)
enalapril 2.5mg p.o.(n=24)	68 (7)	10/12/2	97 (11)	8.2 (11.8)	138 (4.4)	93 (57)	(16/3/3/0/2)
perindopril 2mg p.o (n=24)	69 (8)	8/16/0	94 (11)	5.3 (7.1)	140 (3.2)	93 (44)	(15/3/3/2/1)
enalaprilat 1.5mg i.v.(n=12) (0.25mg/hour for 6 hours)	70 (6)	2/10/0	99 (15)	4.2 (3.7)	141 (2.4)	100 (43)	(8/2/0/1/1)
perindoprilat 1mg i.v.(n=12) (0.167mg/hr for 6 hours)	69 (6)	5/7/0	89 (10)	4.5 (4.7)	140 (3.3)	85 (40)	(7/2/1/1/1)
perindoprilat 0.167mg i.v.(n=12) (0.167mg/hour for 1 hour)	66 (8)	4/8/0	91 (12)	6.8 (8.4)	138 (3.7)	90 (41)	(10/0/1/1/0)
quinapril 2.5mg p.o.(n=12)	76 (8)	8/4/0	95 (12)	2.5 (1.6)	141 (3.1)	62 (18)	(8/2/2/0/0)
placebo (n=36)	71 (8)	5/19/2	92 (14)	4.4 (2.7)	138 (2.5)	96 (69)	(24/5/3/3/1)

AC = alcohol related cardiomyopathy; D = idiopathic; HT = hypertension; IHD = ischaemic heart disease; CHF = congestive heart failure; MAP = mean arterial pressure; NYHA = New York Heart Association; PRA = plasma·renin activity; V = valvular heart disease.

PD response.

8.2.5 Pharmacodynamic/Pharmacokinetic modelling

The parameters estimated for each individual obtained in the above pharmaco-
kinetic analysis were used to determine whether the relationship between pharmacokinetic
data and the haemodynamic response was best described by a linear (where effect depends
upon plasma drug concentration) or sigmoid (where a maximum effect is attained)
concentration/ effect model. Models were again fitted using STATIS and assessed by the
residual sum of squares and Akaike Information Criterion.

Direct PD/PK modelling was carried out using the software package Minitab
(Minitab Inc, PA, USA). A number of the assessed parameters were not normally
distributed and non-parametric methods have been used to describe results. All confidence
intervals (C.I.) are at the 95 % level. Thus a result is significant at the 5 % level if the CI
does not contain 0.

Three kinetic models of the pharmacodynamic effect were compared. In the first a
linear relationship was proposed to relate the mean arterial blood pressure response (E) to
the measured drug concentration [c];

$$E = A.[c] + B,$$

where A and B are the slope and the baseline effect respectively. Two non-linear models
were proposed:

a Langmuir model

$$E = \frac{[c].E_{max}}{[c] + [c_{50}]} + B,$$

and the more general Hill model
$$E = \frac{[c]^A.E_{max}}{[c]^A + [c_{50}]^A} + B,$$
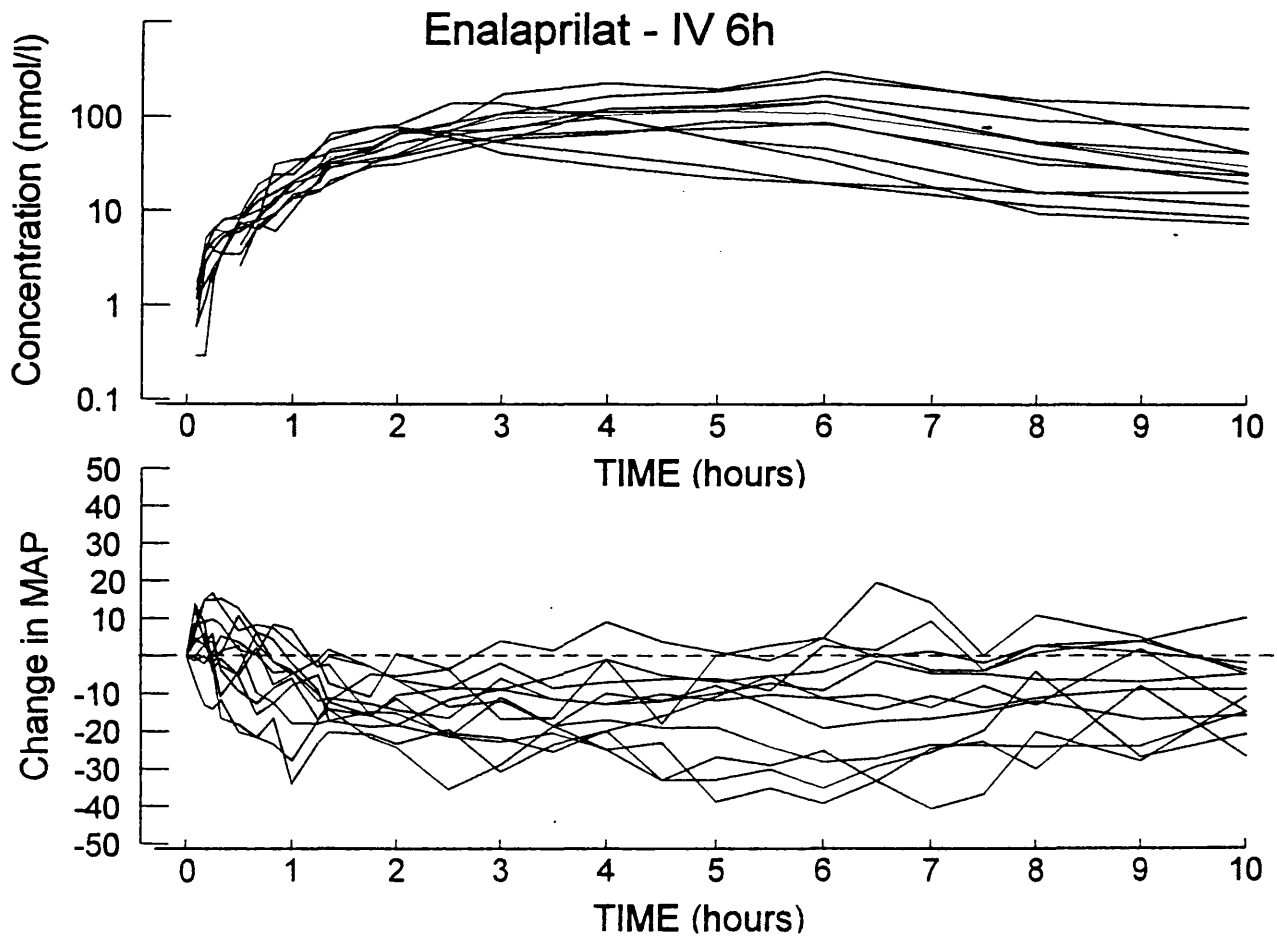
Figure 8.1:
Individual drug (enalaprilat) and MAP time profiles following intravenous infusion of enalaprilat at a constant rate of 0.25mg/hr for 6 hour in patients with mild to moderate CHF.

MAP=mean arterial blood pressure
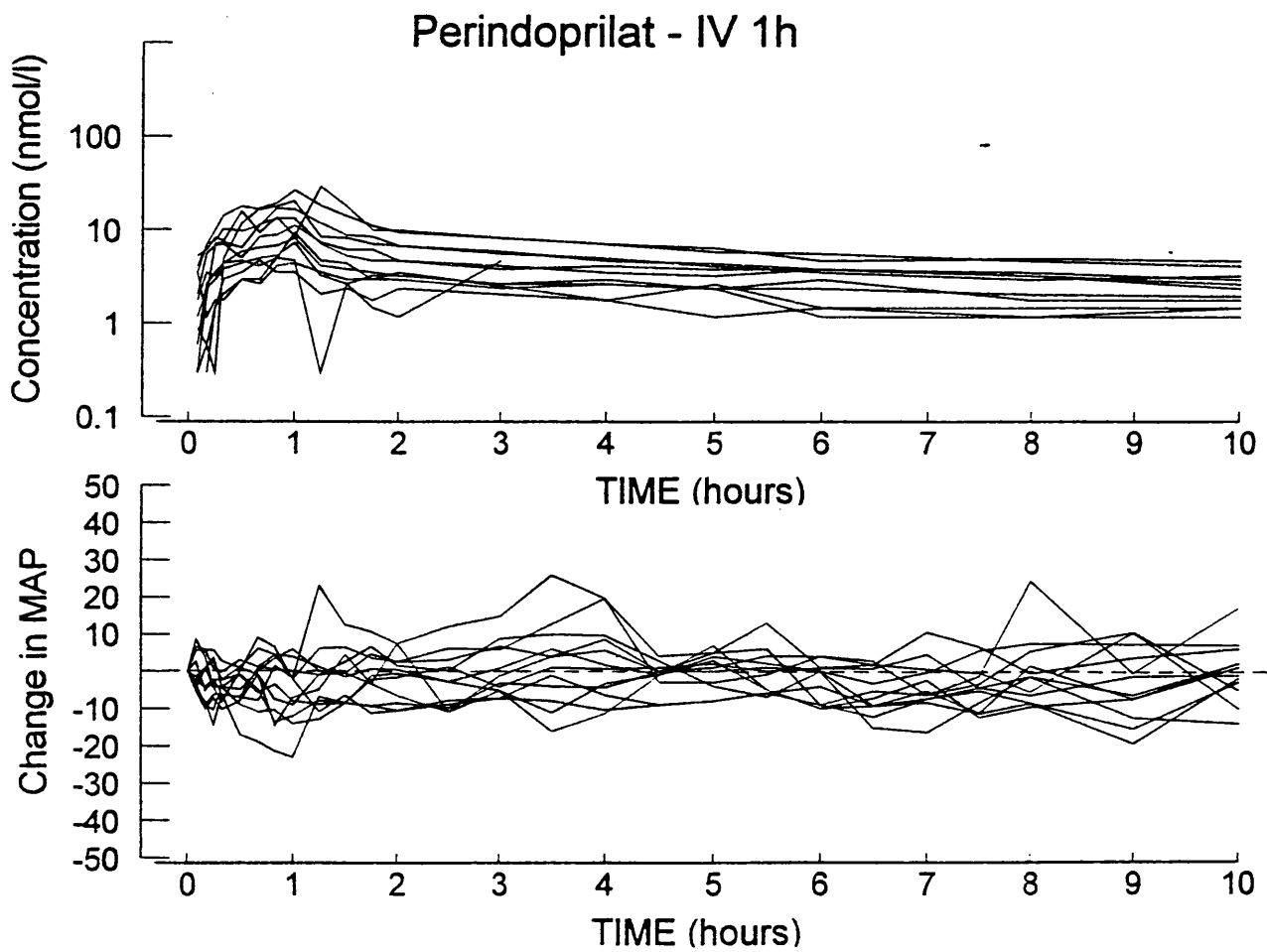
Figure 8.2:
Individual drug (perindoprilat) and MAP time profiles following intravenous infusion of perindoprilat at a constant rate of 0.167mg/hr for 1 hour in patients with mild to moderate CHF.

MAP=mean arterial blood pressure
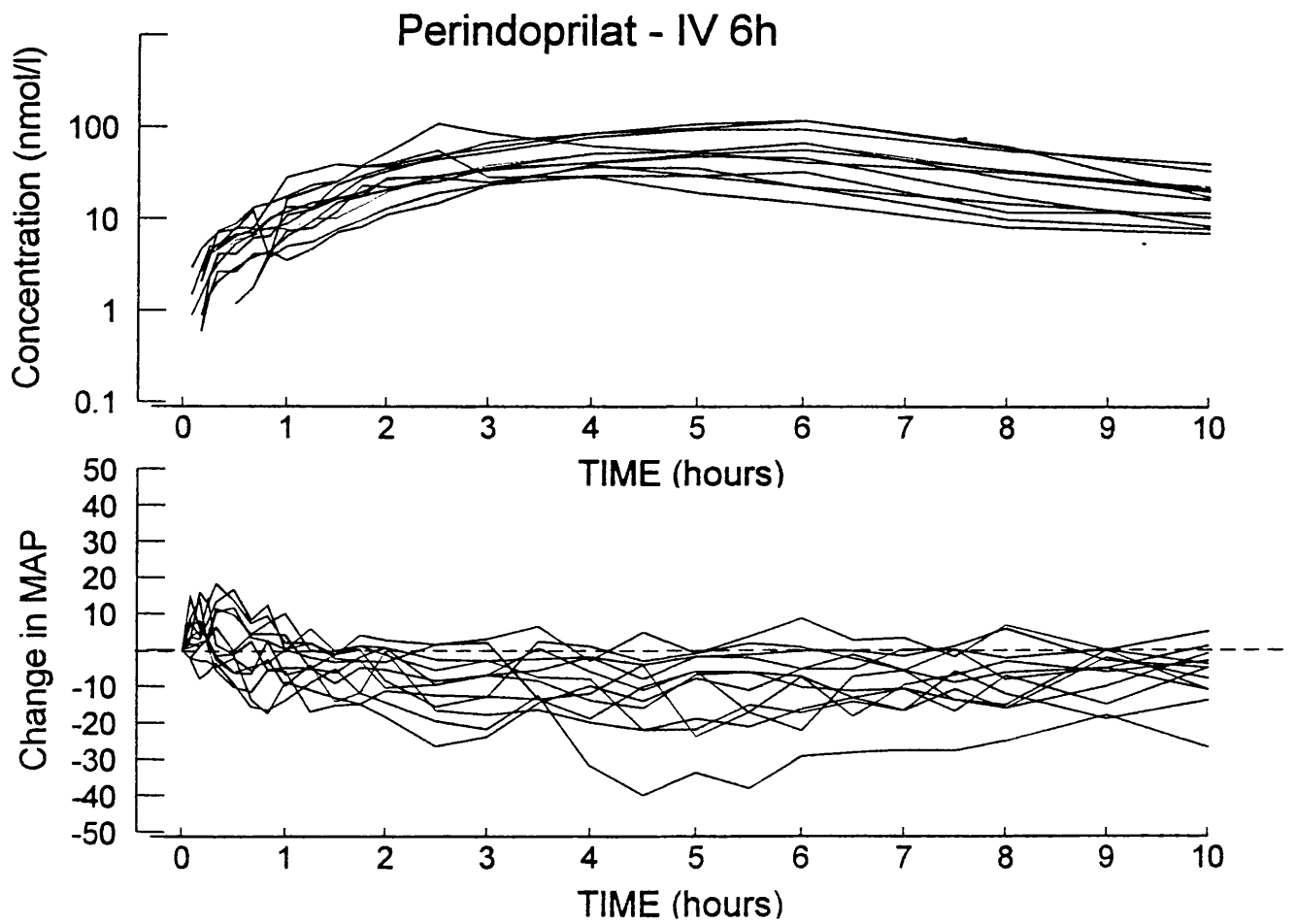
Figure 8.3:
Individual drug (perindoprilat) and MAP time profiles following intravenous infusion
of perindoprilat at a constant rate of 0.167mg/hr for 6 hours in patients with mild to
moderate CHF.

MAP=mean arterial blood pressure

where Emax is the maximum effect, c_{50} is the concentration at which 50 % Emax occurs,

B is the baseline effect and A is constant.

8.2.6 Influence of physiological variables

The relationship was investigated between a number of baseline physiological variables and the magnitude of the blood pressure response, as assessed directly by the AUC10 and indirectly by the slope of the PD/PK relationship (vide infra). For the categorical variables gender (male/female), cardiac rhythm (sinus rhythm, other rhythm), aetiology of CHF (ischaemic heart disease/hypertensive heart disease/alcohol related/ valvular heart disease/idiopathic) and NYHA class (II/ III+IV), the mean arterial pressure responses were compared and 95% confidence intervals calculated for the differences in medians between groups for each variable. Patients with NYHA class III and IV CHF were considered together in view of the small number of patients with class IV disease. The strength of association with the MAP response for the baseline value of each continuous variable (age, baseline values of mean arterial pressure, diuretic dose, ACE activity, plasma renin activity, and each of serum sodium, potassium and creatinine concentrations) was quantified using the Spearman rank correlation coefficient. In addition stepwise linear regression analysis and best-subsets regression analysis were used to determine whether the pharmacodynamic response could be predicted from some subset of the above information. Statistical analysis was carried out using Minitab.

8.3 RESULTS

8.3.1 General

Summary statistics for baseline clinical and laboratory features of each treatment group are shown in Table 8.2. Initiation of study therapy was in no case associated with

symptomatic hypotension. In 10 patients randomised to receive 6 hour infusions, study treatment was stopped prematurely due to a fall in mean arterial pressure of > 30% of baseline, as was stipulated in the study protocol. All remained in the study and haemodynamic data were analysed on an 'intention to treat' basis. One patient, a 74 year old female who had received oral placebo as study treatment, was unable to tolerate subsequent open ACE inhibitor therapy due to symptomatic hypotension after the first dose. Plots of plasma drug concentration (diacid metabolite) against time, together with placebo- and baseline-corrected change in MAP against time for each treatment are shown in Figure 8.1 - 8.6.

8.3.2 Pharmacokinetic analysis

8.3.2.1 Intravenous dosing

Twelve patients received intravenous enalaprilat, 0.25 mg/hr for 6 hours; 5 of the infusions were terminated prematurely at between 2 and 5 hours due to excessive falls in MAP of > 30 % of baseline (Appendix 1.1). A total of 24 patients were given intravenous perindoprilat: 12 received 0.167 mg/hr for 1 hour and 7 received 0.167 mg/hr for 6 hours. Five further 6-hour infusions were terminated at between 2.5 and 5 hours due to excessive falls in mean arterial pressure of > 30 % of baseline (Appendix 1.1).

Pharmacokinetic analysis was carried out for each subject on the basis of the total dose of drug administered in each individual. One and two compartment models with first order elimination were fitted to the intravenous infusion data using the computer package STATIS. In the case of intravenous enalaprilat the data was adequately described by the one compartment model (Figure 8.1). Analysis of the residual (observed-predicted vs. predicted data) concentration plots suggested that in some cases a 2 compartment model
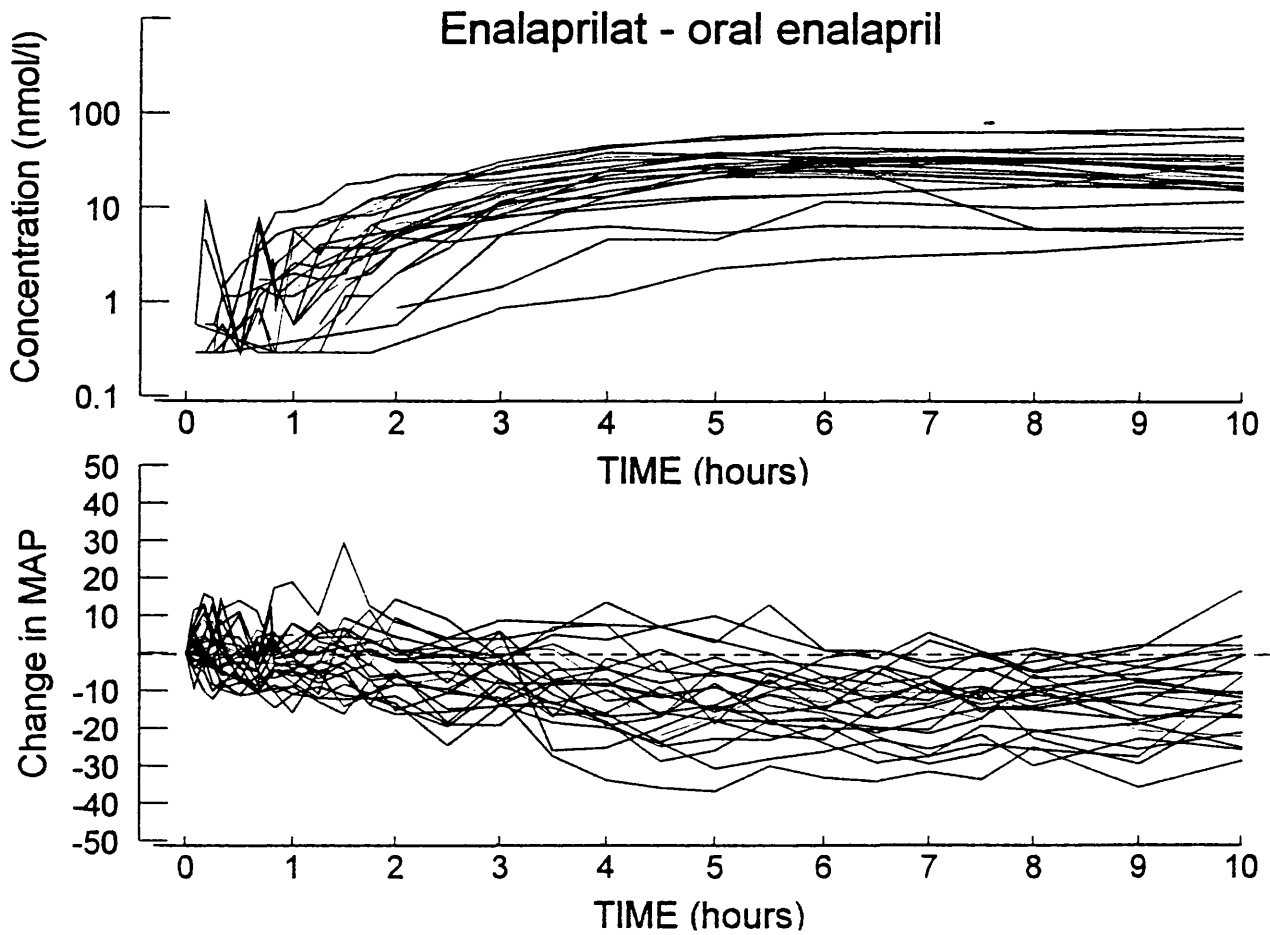
Figure 8.4:
Individual drug (enalaprilat) and MAP time profiles following a single dose of enalapril
2.5mg p.o. in patients with mild to moderate CHF.

MAP=mean arterial blood pressure
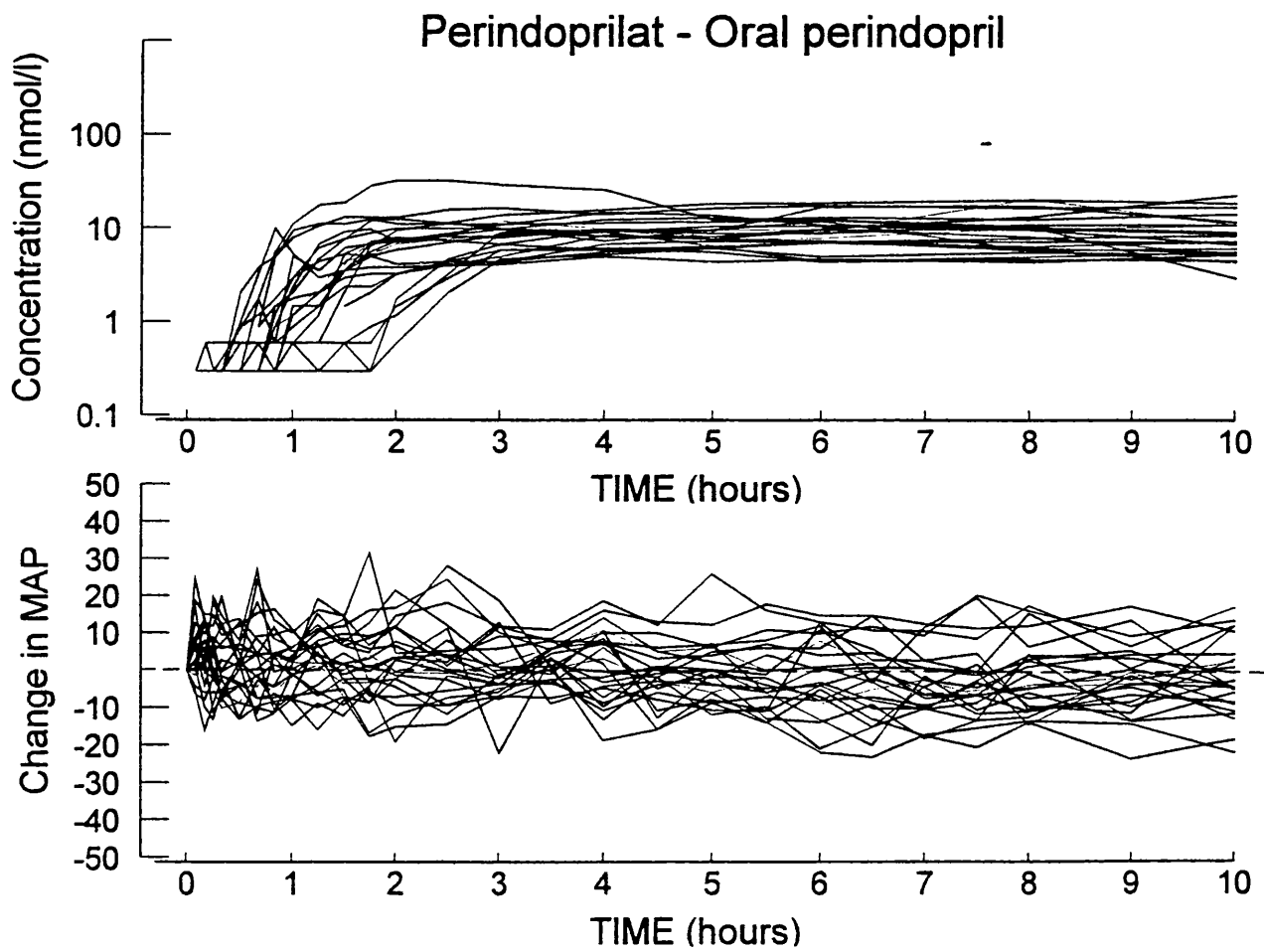
Figure 8.5:

Individual drug (perindoprilat) and MAP time profiles following a single dose of
perindopril 2mg p.o. in patients with mild to moderate CHF.

MAP=mean arterial blood pressure
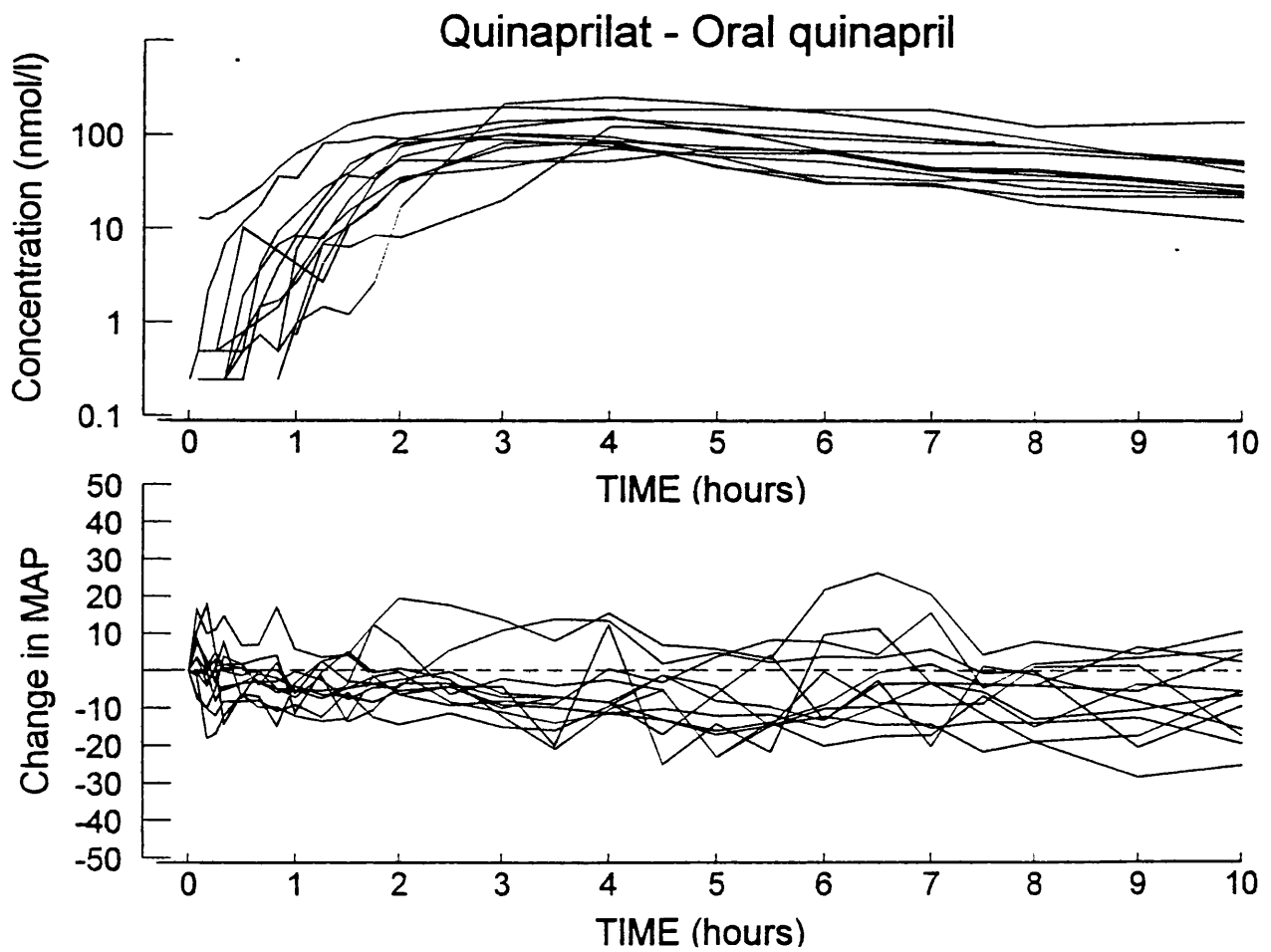
Figure 8.6:
Individual drug (quinaprilat) and MAP time profiles following a single dose of
quinapril 2.5mg p.o. in patients with mild to moderate CHF.

MAP=mean arterial blood pressure

was more valid. With only 3 data points after the end of the 6-hour infusion, the parameters of the model could not be estimated and superiority of the 2 compartment model could not be assumed.

In contrast it was clear from the 1-hour infusion data that the pharmacokinetics of perindoprilat were better described by a 2 compartment model (Figure 8.2) and the formal analysis confirmed this. Data from the 6-hour infusions again suggested that in some cases a 2 compartment model was more valid. Once again there were insufficient data points after the end of the infusion to estimate the parameters of the 2 compartment model (Figure 8.3).

The estimated individual values for the elimination rate constant (Ke) and volume of distribution (Vd) together with the RSS, AIC and SIC for intravenous dosing with enalaprilat and perindoprilat are shown in Appendix 1.2 and 1.3 respectively.

8.3.2.2 Oral dosing

The formation, disposition and elimination of each active diacid metabolite ACE inhibitor was modelled using a one-compartment model and first-order elimination. From the profiles of concentration against time it was evident that it was appropriate to fit a lag phase to model formation of the metabolite. Zero-order and first-order methods were compared when modelling drug absorption and conversion to the active metabolite. In the case of enalapril (Figure 8.4) and quinapril (Figure 8.6) a zero-order process was more appropriate in the majority of cases. Residual plots again suggested that a 2-compartment model may be more appropriate in a number of individuals but there were insufficient data points after the end of the phase of drug absorption and conversion to estimate the model parameters. For oral enalapril, individual parameter estimates of Ke and Vd, together with

AUC10

	Perindoprilat			Enalaprilat		Quinaprilat
mmHg.h	Oral	iv 1h	iv 6h	Oral	iv 6h	Oral

95% CI for median   -37 to 23   -55 to 14   -116 to -31   -120 to -60   -174 to -49   -92 to -11
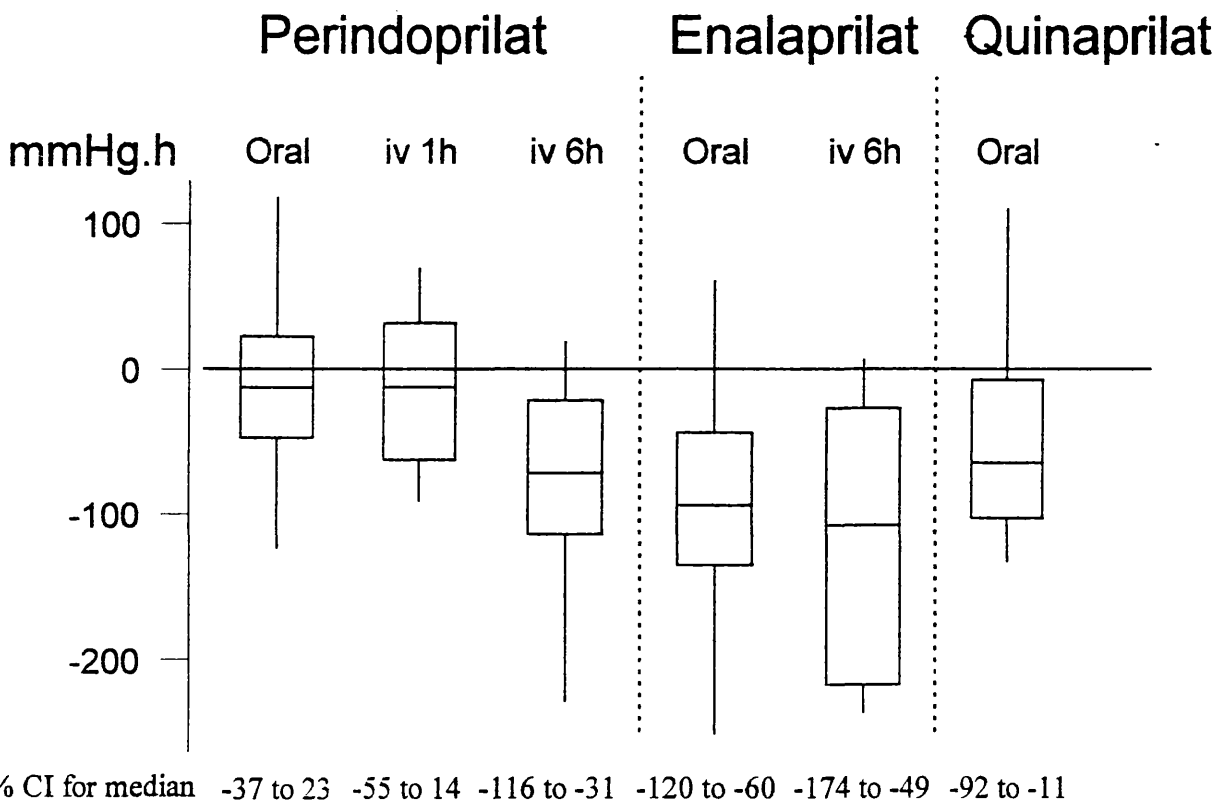
Figure 8.7:
Summary plots showing median, quartiles and range of the pharmacodynamic
response, as assessed directly from the AUC10 of the pharmacodynamic/
pharmacokinetic relationship, following a single dose of ACE inhibitor in patients with
mild to moderate CHF.

SLOPE

Enalaprilat   Perindoprilat Quinaprilat

95%CI for median -0.69 to .08   -0.39 to -0.11   -0.25 to -0.07
                       -1.10 to -0.27   -0.52 to -0.29   -0.08 to 0.01
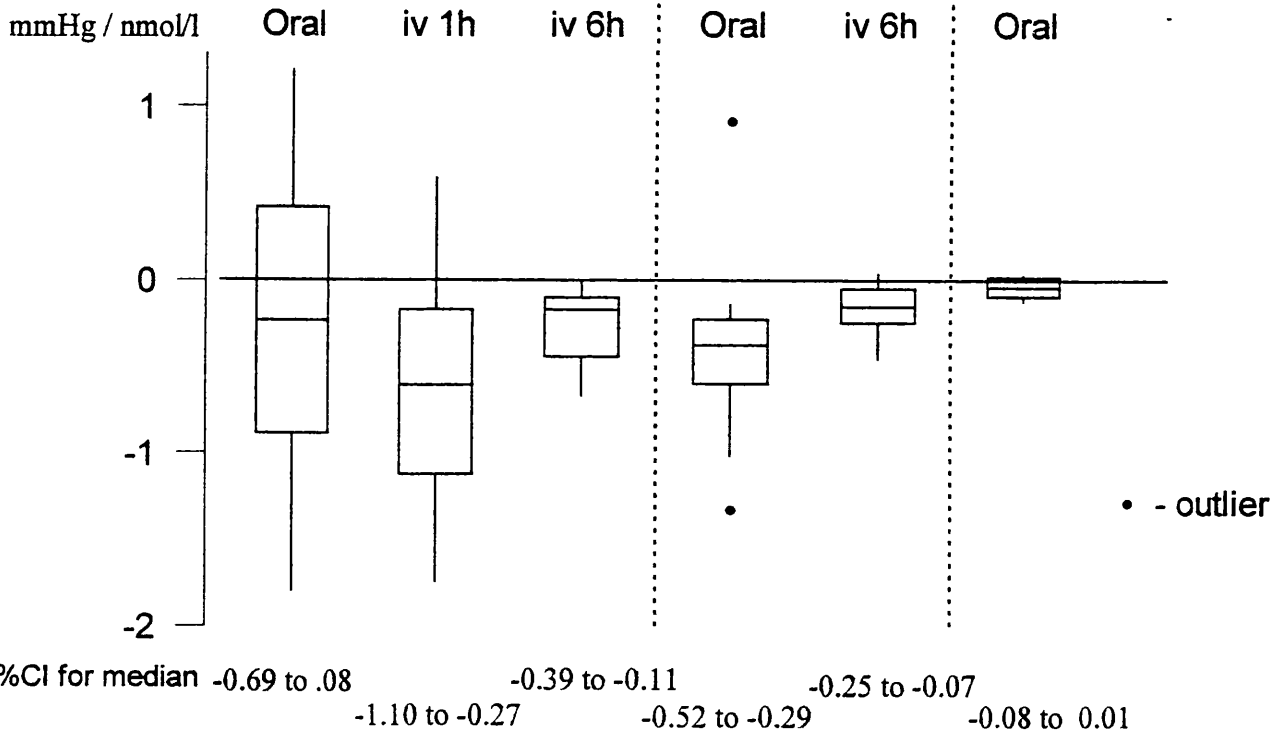
Figure 8.8:
Summary plots showing median, quartiles and range of the pharmacodynamic
response, as assessed indirectly from the slope (β-coefficient) of the pharmacodynamic
/ pharmacokinetic relationship, following a single dose of ACE inhibitor in patients
with mild to moderate CHF.

RSS, AIC and SIC values are shown in Appendix 2.1 and 2.2 for a 1-compartment model with zero-order absorption and a 1-compartment model with 1st order absorption respectively. Corresponding results for oral quinapril are shown in Appendix 3.1 and 3.2 respectively.

There was much greater variation in the data as regards oral perindopril (Figure 8.5). In only 13 of the 24 individuals could a 1-compartment model with first order elimination be fitted to the data. In two patients, drug concentration was still increasing at 10 hours. Individual pharmacokinetic parameter estimates for oral perindopril are shown in Appendix 4.1 and 4.2 for a 1 compartment model with zero-order and first order absorption respectively.

8.3.3 Pharmacodynamic analysis

Summary plots showing the median, quartiles and range for the AUC_{10} are shown for each drug treatment in Figure 8.7. Enalaprilat oral (95% C.I. for median -120 to -60 mmHg.h), enalaprilat i.v. (95% C.I. for median -174 to -49 mmHg.h), perindoprilat 1 mg i.v. over 6 hours (95% C.I. for median -116 to -31 mmHg.h) and quinaprilat (95% C.I. for median -92 to -11) produced a significant fall in mean arterial pressure as estimated by AUC_{10}. With perindoprilat given as 0.167 mg i.v. over 1 hour (95% C.I. for median -55 to 14 mmHg.h) or as oral perindopril (95% C.I. for median -37 to 23 mmHg.h), the median AUC_{10} could not be shown to be different from zero.

8.3.4 Pharmacodynamic/pharmacokinetic modelling

Where the plot of pharmacodynamic effect of a drug against time lags behind that of plasma drug concentration against time, the plot of drug concentration against effect will show an anti-clockwise hysteresis loop. If the data showcompartment requires to be

Table 8.3

Relationship between categorical variables (gender (male/female), New York Heart Association (NYHA) functional class (II/III+IV), and cardiac rhythm (sinus/other)) and MAP response as assessed by β-coefficient (mmHg/ nmol.l^{-1}) of pharmacodynamic/pharmacokinetic relationship.

		Enalaprilat	Perindoprilat	Quinaprilat	All treatments
Gender	(1) Male	-0.31	-0.28	-0.06	-0.25
	(2) Female	-0.23	-0.43	0.01	-0.18
	(1) - (2)	-0.04	0.03	-0.07	-0.04
	95% CI	-0.20-0.20	-0.38-0.47	-0.13-0.01	-0.18-0.14
	p-value (Mann-Whitney)	0.72	0.92	0.10	0.58
NYHA	III + IV	-0.23	-0.39	-0.03	-0.22
	II	-0.38	-0.32	-0.04	-0.24
	(III + IV) - II	0.17	0.06	0.00	0.04
	95% CI	-0.01-0.34	-0.36-0.51	-0.10-0.10	-0.12-0.21
	p-value (Mann-Whitney)	0.07	0.73	0.93	0.69
Cardiac rhythm	(1) Sinus	-0.24	-0.16	0	-0.16
	(2) Other	-0.33	-0.61	-0.06	-0.35
	(1) - (2)	0.09	0.47	0.04	0.17
	95% CI	-0.07-0.28	0.06-0.95	-0.05-0.10	0.02-0.39
	p-value (Mann-Whitney)	0.26	0.02	0.38	0.03

included in the model to accomodate the delay between profiles. The modelling approach

detailed in section 8.2.5 showed that the s such a loop then an effect

pharmacodynamic/pharmacokinetic (PD/PK) relationship was best described by a simple

linear model with a baseline error term, without the need to fit an effect compartment.

This was confirmed by the formal analysis. Thus a direct linear relationship between the

pharmacodynamic effect and plasma drug concentration was derived:

$$\text{MAP}ij = \alpha i + \beta i \ cij ,$$

where MAPij and cij are mean arterial pressure (baseline and placebo corrected) and

plasma drug concentration for individual i at time j, and αi and βi are estimated intercept

and slope of the fitted line for individual i. The estimated slope (i.e. the β-coefficient) of

the relationship was taken as an indirect measure of the magnitude of the relationship

between the pharmacodynamic response and observed drug concentration, ie the PD/PK

relationship.

Individual values for the estimated slope and intercept of the regression line

relating fall in MAP to plasma drug concentration are shown in Appendix 5.1. Summary

plots showing the median, quartiles, range and outliers (> 1.5 interquartile range from the

quartiles) for the β-coefficient of the PD/PK relationship, together with 95% confidence

intervals for the median, are are shown in Figure 8.8. It is clear from the plots that for

enalaprilat oral (95 % CI for median slope -0.52 to -0.29 mmHg/ nmol.l^{-1}) and enalaprilat

i.v. (95 % CI for median slope -0.25 to -0.07 mmHg/ nmol.l^{-1}) there was a negative

estimate of slope, indicating a direct relationship between enalaprilat concentration and fall

in MAP. In keeping with the pharmacokinetic data the relationship of plasma drug

concentration to blood pressure response with perindoprilat was more variable. There was

a direct relationship between concentration and effect for i.v. administration of

Table 8.4

Relationship between categorical variables ((gender (male/female), New York Heart Association (NYHA) functional class (II/III+IV), and cardiac rhythm (sinus/other)) and MAP response as assessed by AUC_{10} (mm Hg.h) of MAP/time profile.

		Enalaprilat	Perindoprilat	Quinaprilat	All treatments
Gender	(1) Male	-71.4	-13.7	-95.8	-43.4
	(2) Female	-100.9	-59.2	-28.7	-75.6
	(1) - (2)	38.8	37.8	-50.3	27.6
	95% CI	27.2-83.5	3.8-69.4	-160.9-274	-5.2-58.1
	p-value (Mann-Whitney)	0.26	0.04	0.19	0.09
NYHA	III + IV	-97.7	-27.4	-47.0	-63.2
	II	-0.90	-19.4	-70.7	-50.2
	(III + IV) - II	-7.7	-4.2	17.4	-3.12
	95% CI	-70.4-53.2	-40.8-35.3	-110.6-104.5	-34.6-29.1
	p-value (Mann-Whitney)	0.88	0.81	0.67	0.86
Cardiac rhythm	(1) Sinus	-65.9	-27.4	-67	-36.1
	(2) Other	-105.9	-7.9	-64.6	-75.6
	(1) - (2)	41.7	1.5	-29.6	29.2
	95% CI	-10.7-94.5	-43.6-40.7	-108.8-67.2	0.02-0.39
	p-value (Mann-Whitney)	0.12	0.93	0.69	0.07

perindoprilat over both 6 hours and 1 hour (95% C.I. for median slope -0.39 to -0.1 and

-1.1 to -0.27 mmHg/ nmol.l^{-1} respectively). In contrast for perindoprilat given as oral

perindopril there was no evidence of a direct relationship between concentration and effect

(95% C.I. for median slope -0.69 to 0.08 mmHg/ nmol.l^{-1}). There was however no

difference between the oral perindopril and intravenous perindoprilat preparations, the

95% confidence intervals for a difference between medians (oral-intravenous) being -0.30

to 0.61 mmHg/ nmol.l^{-1}.

Relatively higher plasma drug concentrations in the face of broadly similar mean

blood pressure responses were achieved with oral quinapril than with other treatments.

Thus the estimated slopes defining the PD/PK relationship were smaller in magnitude than

with other preparations. The 95% C.I. for the median slope were -0.08 to 0.01 mmHg/

nmol.l^{-1}.

It is evident from the results that the response to intravenous administration of

ACE inhibitor is more consistent than to oral therapy. Although for treatments other than

enalapril (oral or intravenous) no consistent relationship was observed between plasma

drug concentration and effect, within each treatment group a close relationship between

plasma drug concentration and blood pressure response was observed for a number of

individual patients including those receiving oral therapy.

8.3.5 Influence of physiological covariates

Both the direct pharmacodynamic response, as measured by the AUC10, and the

indirect PD/PK response, as measured by the slope of the linear model, were considered

when determining which factors may be related to the blood pressure response. As the

distribution of values for some of the variables was skewed, the Spearman rank correlation

Table 8.5

Spearman rank correlation coefficient (RCC) for the relationship between the value of continuous physiological variables and fall in MAP after a single dose of ACE inhibitor. Results for enalaprilat and perindoprilat represent combined results of individual studies.

Physiological variable									
Treatment		Na^+	K^+	Cr	MAP	ACE	PRA	Age	Diuretic
Quinaprilat	AUC_{10}	-0.494	-0.549	-0.266	-0.011	0.231	-0.017	-0.210	-0.052
	β-coeff	-0.653	-0.452	-0.168	0.074	0.025	0.009	-0.021	-0.190
Enalaprilat	AUC_{10}	-0.042	0.014	-0.005	-0.380	-0.200	-0.276	-0.010	-0.305
	β-coeff	0.183	-0.337	0.046	0.064	0.036	-0.294	-0.020	-0.078
Perindoprilat	AUC_{10}	0.011	0.074	0.370	0.115	0.093	-0.090	-0.188	-0.051
	β-coeff	0.122	0.082	0.078	-0.354	-0.098	0.050	-0.145	0.212
All treatments	AUC_{10}	-0.125	-0.038	0.009	0.158	-0.177	-0.025	-0.123	-0.164
	β-coeff	0.041	0.160	-0.070	0.068	-0.143	-0.009	-0.112	0.019

coefficient was used to assess the strength of the association of each variable with the MAP response. When categorical variables were considered, patients with abnormal cardiac rhythm had a greater fall in MAP as assessed by the β-coefficient of the PD/PK relationship (95% C.I. for difference between medians, 0.02 - 0.39, p=0.03). Neither gender (95% C.I. -0.18 to -0.14, p=0.58), nor NYHA class (95% C.I. -0.12 to 0.21, p= 0.69) showed any correlation with blood pressure response (Table 8.3).

When AUC_{10} was considered, no categorical variable could be shown to be associated with the fall in MAP (Table 8.4). The statistically strongest relationship was again with abnormal cardiac rhythm (95% C.I. for difference between medians, -3.87 to 58.0, p=0.07). Spearman rank correlation coefficient values for β-coefficient and AUC_{10} for each continuous variable for each ACE inhibitor drug considered alone and for treatments combined are given in Table 8.5. There was no consistent relationship between any single covariate and either measure of blood pressure response. Although in each study significant correlations between covariates and slope were identified, a number of such results can be expected by chance and were not reproduced when studies were considered together.

To determine whether the blood pressure response can be predicted from a combination of baseline variables, forward stepwise regression analysis was carried out using age, gender, cardiac rhythm, New York Heart Association functional class, diuretic dose, serum ACE activity, plasma renin activity, and serum sodium, potassium and creatinine concentrations as predictor variables with AUC_{10} and β-coefficient as response variables. Where the data were skewed, logarithmic and reciprocal transformations were also considered. The regression analysis was performed using all active treatments considered together and also for each drug treatment individually. The proportion of

179

Table 8.6
Stepwise forward regression analysis for MAP response as assessed by AUC_{10} and
β-coefficient of pharmacodynamic/pharmacokinetic relationship.

	AUC_{10}			β-coefficient			
Variables in model	Adjusted R^2	F	p	Variables in model	Adjusted R^2	F	p
MAP	5.8	6.68	0.02	cardiac rhythm	5.8	6.69	0.02
MAP +PRA	10.6	5.88	0.03	cardiac rhythm +PRA	6.7	1.92	0.34
MAP +PRA +1/Cr	13.6	4.17	0.09	cardiac rhythm +PRA +diuretic dose	7.9	2.19	0.28
MAP +PRA +1/Cr +Age	14.4	1.79	0.37	cardiac rhythm +PRA +diuretic dose	8.8	1.96	0.33
MAP +PRA +1/Cr +Age +Drug	23.6	6.32	<0.01	cardiac rhythm + PRA +diuretic dose +MAP + Drug	14.2	7.35	<0.01

R^2 = percentage variability in response explained by the value predictor variable(s). The R^2
value will increase as more predictor variables are added to the model. Adjusted R^2 takes
account of the number of variables in the model.

ACE = Angiotensin converting enzyme activity; CR = serum creatinine concentration; diuretic
= daily diuretic dose (mg frusemide or equivalent); MAP = mean arterial pressure; PRA =
plasma renin activity.

variability (R^2) in mean arterial pressure response explained by each predictor variable was used to compare contending models.

The results for each individual treatment were similar to the overall analysis and are not presented. The subsets of predictors containing between 1 and 4 variables are shown in Table 8.6. In no case did the inclusion of a fifth variable other than the nature of the ACE inhibitor increase the value of the adjusted R^2. The results show that less than 15% of the variability in mean arterial pressure response can be explained on the basis of the considered baseline factors. When the ACE inhibitor agent is also considered, the adjusted R^2 values increase to 23.6% and 14.2% for AUC_{10} and β- coefficient respectively. The models including drug as a variable gave an improved fit (F=6.32, p<0.01) but still left over 75% of the variability in response unexplained. Best subsets regression analysis gave similar results.

Baseline physiological variables were compared within the population of 24 patients who received 6-hour intravenous infusion therapy. When the 10 patients in whom infusion was terminated prematurely due to the magnitude of the blood pressure fall were compared to the remaining 14 who received intravenous treatment, an association emerged between the magnitude of the blood pressure response and high baseline plasma renin activity (p=0.03, Mann-Whitney U-test, Table 8.7).

8.4 DISCUSSION

This analysis represents the most systematic analysis to date of the first-dose blood pressure response on initiation of ACE inhibitor therapy in patients with mild to moderate CHF. All patients were studied using a standard protocol of drug administration, blood sampling and supine haemodynamic monitoring. Current clinical practice was

reflected in that, for oral therapy, each agent was administered in its recommended starting dose in CHF and on a background of 24-48 hours diuretic withdrawal. The parameters studied represent the easily identifiable clinical and physiological variables which are routinely available to physicians prior to starting ACE inhibitor therapy. No consistent relationship could be established between any pre-treatment parameter and the blood pressure response. Even when using the best combination of predictive physiological variables (baseline values of mean arterial pressure, plasma renin activity, creatinine concentration, age and the ACE inhibitor agent), less than 25% of the variability in blood pressure fall could be explained on the basis of baseline information. The results indicate that the first-dose hypotensive response cannot be predicted from baseline clinical or physiological variables in patients with mild to moderate CHF.

A much more consistent pharmacodynamic response was seen where inter-individual pharmacokinetic differences are minimised by intravenous dosing. For oral therapy, interindividual differences in drug metabolism appear to explain pharmacodynamic differences, the blood pressure response largely reflecting the plasma concentration of active diacid achieved.

While various parameters have been suggested to predict the likelihood of first-dose hypotension on initiation of ACE inhibitor therapy, such assertions are based largely on evidence from studies in patients with severe CHF (Cleland et al 1985, Hodsman et al 1983, Packer et al 1986, Packer 1989). While hyponatraemia and high doses of loop diuretic are associated with first-dose hypotension in severe heart failure (Packer 1989), studies of the determinants of the blood pressure response in patients with mild to moderate CHF are lacking. In a study of the acute effects of oral captopril in 36 patients with mild to moderate CHF, neither plasma sodium nor diuretic dose were predictive of

the blood pressure response (Motwani et al 1994). The present analysis extends these results to include oral perindopril and quinapril, and to enalaprilat and perindoprilat given intravenously. In spite of a high average daily diuretic dose and wide range of dose in the present population, there was no clear relationship to the blood pressure response.

In the study of Motwani et al, the fall in plasma noradrenaline and angiotensin II levels in response to initiation of ACE inhibitor therapy correlated with the fall in blood pressure (Motwani et al 1994). It is perhaps a limitation of the present study that no attempt was made to relate the blood pressure response to baseline levels of atrial natriuretic factor, noradrenaline or angiotensin II. However in the study of Motwani none of these was predictive of the blood pressure response to initiation of therapy. Moreover, information on the level of these neurohormones is not available to the majority of physicians in routine practice, and the present study was designed to assess the predictive value of routinely available parameters. For similar reasons no attempt was made to relate blood pressure response to left ventricular ejection fraction. In the absence of information on ejection fraction, the identification of abnormal cardiac rhythm as a predictor of the blood pressure response may indicate a subgroup of patients with more severe CHF at greater risk of a fall in blood pressure. Similarly the identification of baseline plasma renin activity as a predictor of MAP response in those patients receiving intravenous therapy suggests an important role for activation of the renin-angiotensin system in the magnitude of the blood pressure response in patients with mild to moderate CHF. This is in keeping with the correlation between the magnitude of the fall in angiotensin II concentration and blood pressure response seen in the study of Motwani.

In the present analysis the extent of the blood pressure fall with acute ACE inhibition was related to the level of pre-treatment blood pressure, larger falls being seen

Table 8.7

Comparison of of baseline physiological values (Mann-Whitney U-test for comparison of medians) in patients receiving 6-hour infusion therapy. Patients were divided according to a fall in MAP of > 30% (M_S, n=10) or < 30% (M_{NS}, n=14) from baseline.

Variable	Median (M_{NS})	Median (M_S)	Estimated (M_{NS}-M_S)	95 % C.I. M_{NS}-M_S	p-value
PRA	0.965	5.090	-2.905	-5.37 to -0.73	0.03
ACE	22.45	21.35	1.7	-4.3 to 6.6	0.64
MAP	97.5	89.0	5.0	-8.0 to 18.0	0.40
Age	68.5	70.5	-2.0	-8.0 to 4.0	0.70
Na^+	140	139.5	1.5	0.0 to 4.0	0.07
K^+	4.4	4.05	0.1	-0.3 to 0.6	0.77
Cr	104.5	95.0	4.0	-17.0 to 25.0	0.70
Diuretic (mg frusemide/day	80	80	0	-40 to 40	0.75

ACE = Angiotensin converting enzyme activity; Cr = plasma creatinine concentration; MAP = mean arterial pressure; PRA = plasma renin activity.

with higher pre-treatment levels. Although baseline mean arterial pressure was the strongest single determinant of the blood pressure response, only a small fraction, 6-7%, of the variability in response could be explained on this basis. Further to this, during stepwise regression analysis the best subset of baseline variables could explain around only 25% of the variability in blood pressure response. While Motwani was able to explain 74% of the variability in blood pressure response to captopril, mean arterial pressure was the only baseline parameter in the predictive model, the others being the magnitude of the falls in plasma noradrenaline and angiotensin II levels after initiation of treatment. Pre-treatment mean arterial pressure is a well recognised determinant of the blood pressure response to ACE inhibition in hypertension (Hodsman et al 1983), as is the case for all agents which lower blood pressure.

The incidence of first-dose hypotension varies according to patient populations and reporting procedures, some suggesting 10-15% (Hodsman et al 1983), others 30-40% (Packer et al 1986). In patients with CHF the incidence of symptomatic hypotension has been reported as approximately 5-10% (The CONSENSUS Trial Study Group 1987, AIRE Study Group 1993); between 2 % and 5% withdraw from treatment as a result. The occurrence of first dose hypotension was considered a sufficient problem to warrant reduction of the starting dose of enalapril in an early trial of ACE inhibition in CHF (The CONSENSUS Trial Study Group 1987). In addition, mortality was higher among those patients who developed first-dose hypotension after initiation of therapy with enalapril in the CONSENSUS II study (Swedberg et al 1992). However the latter study differs from others in that administration of ACE inhibitor was by intravenous infusion and within three days of acute myocardial infarction.

On the basis of the above evidence a variety of protocols have been developed for

the initiation of ACE inhibitor treatment in CHF (McMurray et al 1989). Such procedures

have implications for the patient in terms of time and inconvenience, and for the individual

physician and health services as a whole in terms of allocation of resources. Moreover,

many physicians remain reluctant to start ACE inhibition on the basis of concerns

regarding first-dose hypotension. A recent postal survey of General Practitioners revealed

that 46% expressed some degree of concern regarding potential adverse effects of ACE

inhibition (Houghton & Cowley 1996). Of these, 86% identified hypotension, particularly

on the first dose, as the adverse effect of most concern. This compared to 45% expressing

concerns regarding renal impairment and 9% regarding electrolyte disturbance. Moreover,

GPs expressing such concerns were less likely to have initiated ACE inhibitor therapy for

CHF. Reluctance to implement the findings of the major trials of ACE inhibitors in CHF is

not confined to the primary care setting. Marked variation in the prescription of ACE

inhibition in CHF exists between hospitals (Philben et al 1996). This applies to both

patients with chronic heart failure (Missouris & MacGregor 1996) and those with heart

failure after acute myocardial infarction (Sapsford et al 1996). The latter study surveyed

235 patients with heart failure after MI. In only 20% was ACE inhibitor initiated prior to

discharge from hospital. Again a proportion of physicians identified the possibility of

hypotension as a factor in the non-prescription of ACE inhibitors.

From the above, it is evident that identification of individual patients at high risk of

first-dose hypotension would have therapeutic and economic implications. Moreover,

identification of those at low risk would perhaps allow more widespread prescription of

appropriate therapy. In this context it is perhaps surprising that studies of possible agent-

or dose-related differences in response are rare. Within the dose ranges currently available

there is good evidence that in any individual patient, there is no difference in the

magnitude of blood pressure response to a single dose of either 6.25mg or 25mg of captopril (McLay et al 1992). While it has been suggested that the determinants of the response to chronic ACE inhibition may differ from those determining the acute response (Cleland et al 1987), the available evidence indicates that in patients with CHF the blood pressure response to the first dose of ACE inhibitor is the same as that seen after chronic treatment (Packer et al 1986, McLay et al 1992). In this context, the occurrence of only a single episode of symptomatic hypotension on initiation of therapy in the population of 144 patients studied here with mild to moderate CHF is reassuring.

In summary, the present study indicates that the blood pressure response to the initiation of ACE inhibitor therapy in patients with chronic, stable CHF cannot be predicted from baseline pathophysiological variables. The present lack of consensus on both the factors which constitute a patient at high risk of significant first-dose hypotension and the optimum protocol for the avoidance of the phenomenon in CHF indicate that this is the situation as presently perceived in clinical practice. While the magnitude of blood pressure response is in general related to plasma drug concentration, inter-individual variation in the magnitude and duration of response is high.

CHAPTER 9

CONCLUDING REMARKS

The studies described here demonstrate differences among prodrug ester ACE inhibitor agents in terms of the haemodynamic response to initiation of therapy in patients with CHF. However although the average blood pressure response differs from one agent to another, there is wide inter-individual variability in the response to any one agent. The ranges of responses observed are thus very similar among ACE inhibitor agents.

The apparent transient pressor response to initiation of therapy previously observed was inconsistent in the current studies and indeed was observed with placebo. This phenomenon is likely to be a manifestation of an alerting response associated with the activity of study initiation rather than to the displacement of angiotensin peptides from binding sites. No evidence was found for the putative inter-agent pharmacokinetic differences of steric hindrance on the one hand and "slow, tight binding" on the other. Differences among ACE inhibitors in terms of their binding to plasma and to tissue ACE may explain differences in blood pressure response.

The assay of angiotensin peptide concentrations offered no additional information over and above that provided by assay of plasma ACE in terms of defining the state of activity of the circulating RAS. This may be due to the limitations of the assay in our laboratory including those associated with its application to a large number of samples from patients in a clinical setting. The assay is unlikely to have advantages over assay of plasma ACE activity in standard clinical studies.

The observed inter-individual differences in response to oral therapy appear to be related largely to inter-individual pharmacokinetic differences. These findings do not prove

that plasma ACE inhibition is the mechanism by which ACE inhibitors lower blood pressure. However these studies offer no evidence for inhibition of tissue ACE as the defining action in this regard. The blood pressure response in the current studies was on the whole related to the concentration of ACE inhibitor agent achieved in the plasma.

The analysis described in Chapter 8 represents the largest and most systematic investigation of the relationship of baseline clinical and laboratory variables to the blood pressure response to initiation of ACE inhibitor therapy. The study shows that the blood pressure response can not be accurately predicted using readily available criteria. However the correlation of blood pressure response with baseline plasma renin activity, particularly where interindividual pharmacokinetic differences were minimised by intravenous administration of ACE inhibitor, suggests the importance of activation of the renin angiotensin system in the response.

The ACE inhibitors are currently one of the cornerstones of the treatment of congestive heart failure. However in spite of their proven benefits, their administration to patients for whom they are undoubtedly indicated remains inconsistent. Prescription is limited, to some extent at least, by physicians' concerns regarding the possibility of symptomatic hypotension on initiation of therapy. However the available literature suggests that the phenomenon is infrequent. Moreover, the blood pressure response to the first dose of ACE inhibitor is very similar to that seen with later doses. A symptomatic fall in blood pressure is an infrequent phenomenon in patients with mild to moderate CHF. Although the extent of blood pressure fall can not be predicted with any accuracy, the observed differences among agents may provide alternatives for initiation of therapy in selected patient groups perceived to be at high risk.

REFERENCES

Adamopoulos S, Brunotte F, Thompson CH, Stratton J, Radda GK (1993).
Physical training improves skeletal muscle metabolism in patients with chronic heart failure. J.Am.Coll.Cardiol. 21: 1101-1106.

Adamopoulos S, Piepoli M, Qiang F,. et al (1995)
Effects of pulsed β-stimulant therapy on β adrenoceptors and chronotropic responsiveness in chronic heart failure. Lancet 345: 344-349.

Admiraal PJJ, Derkx, FHM, Danser AHJ, Preterman H, Schalekamp MADH (1990).
Metabolism and production of angiotensin I in different vascular beds in subjects with hypertension. Hypertension 15: 44-55.

Ajayi AA, Campbell BC, Howie CA, Reid JL (1985).
Acute and chronic effects of the converting enzyme inhibitors enalapril and lisinopril on reflex control of heart rate in normotensive man. J.Hypertension 3: 47-53.

Alicandri C, Fariello R, Boni E, Zaninelli A, Castellano M, Beschi M, Rosei EA, Muiesan G (1987).
Captopril versus digoxin in mild-moderate chronic heart failure: a crossover study. J.Cardiovasc.Pharmacol. 9 (Suppl. 2): S61-S67.

Allen IS, Cohen NM, Dhallan RS, et al (1988).
Angiotensin II increases spontaneous contractile frequency and stimulates calcium current in cultured neonatal rat myocytes: insights into the underlying biochemical mechanisms. Circ.Res. 62: 524-534.

Ambrosioni E, Borghi C, Magnani B for the survival of myocardial infarction long-term evaluation (SMILE) study investigators (1995).
The effect of the angiotensin converting enzyme inhibitor zofenopril on mortality and morbidity after anterior myocardial infarction. N.Engl.J.Med. 332: 80-85.

Anand IS, Ferrari R, Kalra GS, Wahi PL, Poole-Wilson PA, Harris PC.
Edema of cardiac origin: studies of body water and sodium, renal function, haemodynamic indexes and plasma hormones in unterated congestive cardiac failure.
Circulation 1989 80: 299-305.

Anderson JL, Gilbert EM, O'connell J, Renlund D, Yanowitz F (1991).
Long-term (2 year) beneficial effects of beta adrenergic blockade with bucindolol in patients with idiopathic dilated cardiomyopathy. J.Am.Coll.Cardiol. 17: 1373-1381.

Anderson S, Rennke HG, Garcia DL, Brenner BM (1989).
Short and long term effects of of antihypertensive therapy in the diabetic rat.
Kidney Int. 36: 526-536.

Anderson S, Bouyones B, Clarey LE, Ingelfinger JR (1990).
Intrarenal renin-angiotensin system in experimental diabetes.

J.Am.Soc.Nephrol.1 :621(Abs)

Anker SD, Swan JW, Volterrani M, Chua TP, Clark AL, Poole-Wilson PA, Coats JS (1997).
The influence of muscle mass, strength, fatiguability and blood flow on exercise capacity in cachectic and non-cachectic patients with chronic heart failure. Eur.Heart J. 18: 259-269.

Arnold SB, Byrd RC, Meister W, et al. (1980).
Long-term digitalis therapy improves left ventricular dysfunction in heart failure. N.Engl.J.Med. 303: 1443-1447.

Baba T, Murabayashi S, Takebe K (1989).
Comparison of the effects of angiotensin converting enzyme inhibitor and calcium antagonist in hypertensive type 2 (non insulin-dependent) diabetic patients with microalbuminuria: a randomised controlled trial. Diabetologia 32: 40-44.

Baker JB, Wilen MM, Body CM, Dinh H, Franciosa JA (1984).
Relation of right ventricular ejection fraction to exercise capacity in chronic left ventricular failure. Am.J.Cardiol. 54: 596-599.

Baker KM, Campanile CP, Trachte GJ, Peach MJ (1984).
Identification and characterisation of the rabbit angiotensin II myocardial receptor. Circ.Res. 54: 286-293.

Bakris GL (1990).
Effects of diltiazem or lisinopril on massive proteinuria associated with diabetes mellitus. Ann.Int.Med. 112: 707-708.

Balcon R, Jewitt DE, Davies JP, et al (1966).
A controlled trial of propranolol in acute myocardial infarction. Lancet 2: 917-920.

Banas JS (1989).
Preliminary haemodynamic report of the efficacy and safety of quinapril in acute and chronic treatment of patients with congestive heart failure.
Angiology 40: 396-404.

Bangdiwala SI, Weiner DH, Bourassa MG, Friesinger GC, Ghali JK, Yusuf S, for the SOLVD investigators (1992).
The Studies of Left Ventricular Dysfunction registry: Rationale, design, methods and description of baseline characteristics. Am.J.Cardiol. 70: 347-353.

Bank N, Lahorra MAG, Aynedjin HS, Schlondorff D (1988).
Vasoregulatory hormones and the hyperfiltration of diabetes.
Am.J.Physiol. 254: F202-F209.

Banning AP, Lewis NP, Northridge DB, Elborn JS, Henderson AH (1995).
Perfusion/ventilation mismatch during exercise in chronic heart failure: an investigation of

circulatory determinants. Br. Heart J. 74: 27-33.

Bayliss J, Norell, MS, Canepa-anson R, Poole-Wilson PA (1985).
Clinical importance of the renin angiotensin system in chronic heart failure: a double-blind comparison of captopril and prazosin. Br.Med.J. 290: 1861-1865.

Begg EJ, Robson RA, Bailey RR, Lynn KL, Frank GJ (1990).
The pharmacokinetics and pharmacodynamics of quinapril and quinaprilat in renal impairment. Br. J. Clin.Pharmacokin. 30:213-220.

Bickerton RK, Buckley JP (1961).
Evidence for a central mechanism in angiotensin induced hypertension.
Proc.Soc.Exp.Biol.Med. 106: 834-836.

Binkley PF, Nuntziata E, Hatton P, Proicou G, Cody RJ (1992).
Flosequinan augments parasympathetic and attenuates sympathetic drive in congestive heart failure: demonstration by analysis of heart rate variability (abstract).
J.Am.Coll.Cardiol. 19: 147A.

Binkley PF, Haas GJ, Starling RC, et al (1993).
Sustained augmentation of parasympathetic tone with angiotensin converting enzyme inhibitor in patients with congestive heart failure. J.Am.Coll.Cardiol. 21: 655-661.

Bjorck S, Nyberg G, Mulec H, Granerus G, Herlitz H, Aurell M (1986).
Beneficial effects of angiotensin converting enzyme inhibition on renal function in patients with diabetic nephropathy. Br.Med.J. 293: 471-474.

Bjorck S, Mulec H, Johnsen SA, Nyberg G, Aurell M (1990).
Contrasting effects of enalapril and metoprolol on proteinuria in diabetic nephropathy.
Br.Med.J. 300: 904-907.

Blumberg AL, Ackerly JA, Peach MJ (1975).
Differentiation of neurogenic and myocardial angiotensin II receptors in isolated rabbit atria. Circ.Res. 36: 719-726.

Blythe WB (1983).
Captopril and renal autoregulation.N.Engl.J.Med. 308: 390-391.

Bonner G, Preis S, Schunk U, Toussaint C, Kaufmann W (1990).
Haemodynamic effects of of bradykinin on systemic and pulmonary circulation in healthy and hypertensive humans. J.Cardiovasc.Pharmacol. 15 (Suppl. 6): S46-S56.

Boots Company (1993).
Warning on flosequinan. Lancet 341: 1146.

Booz GW, Reisprish MJ, Baker KM (1995).
AT2 receptor blockade augments angiotensin II-induced cardiomyocyte hypertrophy.
Hypertension 25: 547.

Bouaziz H, Joulin M, Safar M, Benetos A (1994).
Effects of bradykinin B$_2$ receptor antagonism on the hypotensive effects of ACE inhibition. Br.J.Pharmacol. 113: 717-722.

Boucher R, Saidi M, Genest J (1972).
A new "angiotensin I converting enzyme" system.
In: Genest J, Kiow E, Eds. Hypertension. New York: Springer Verlag. 512-532.

Braun-Menendez E, Fasciolo JC, Leloir LJ, Munoz JM, Taquinin AC (1946).
In: Renal hypertension. Charles C. Thomas, Ed. Springfield, Illinois.

Braunwald E, Mueller HS, Kloner RA, Maroko RR.(1983).
Role of beta adrenergic blockade in in the therapy of patients with myocardial infarction.
Am.J.Med. 74: 113-123.

Brichard SM, Santoni JPh, Thomas JR, Van de Voorde K, Ketelslegers JM, Lambert AE (1989).
Long-term reduction of microalbuminuria after 1 year of angiotensin converting enzyme inhibition by perindopril in hypertensive insulin tretaed diabetic patients.
Diab. Metab. 16: 30-36.

Brilla CG, Pick R, Tan LB, Janicki JS, Weber KT (1990).
Remodeling of the rat right and left ventricles in experimental hypertension.
Circ.Res. 67: 1355-1364.

Brilla CG, Janicki JS, Weber JT (1991).
Cardioreparative effects of lisinopril in rats with genetic hypertension and left ventricular hypertrophy. Circulation 83: 1771-1779.

Bristow MR, Ginsberg R, Minobe W, et al (1982).
Decreased catecholamine sensitivity and beta adrenergic receptor density in failing human hearts. N.Engl.J.Med. 302: 205-211.

Brown JJ, Davies DL, Johnson VW, Lever AF, Robertson JIS (1970).
Renin relationships in congestive cardiac failure, treated and untreated.Am.Heart J. 80: 329-342.

Brown JJ, Casals-Stenzel J, Cumming AMM, et al (1979).
Angiotensin II, aldosterone and arterial pressure: a quantitative approach. Hypertension 1: 159-179.

Buller NP, Poole-Wilson PA. (1990).
Mechanism of the increased ventilatory response to exercise in patients with chronic heart failure. Br. Heart J. 63: 281-283.

Bund SJ, Aalkjaer C, Heagerty AM, Leckie B, Lever AF (1989).
The contractile effects of porcine tetradecapeptide renin substrate in human resistance

vessels: evidence of activation by vascular wall renin and serine proteases.
J.Hypertension 7: 741-746.

Bunnemann P, Fuxe K, Ganten D (1993).
Extrarenal renin systems: the brain. In: Robertson JIS, Nicholls MG, Eds.. The renin-angiotensin system, Volume 1 London, Gower: 41.1-41.17.

Burgraff GW, Parker JO (1975).
Prognosis in coronary artery disease: angiographic, haemodynamic and clinical factors.
Circulation 51: 146-56.

Cabanes LR, Weber SN, Matran R, et al (1989).
Bronchial hyperresponsiveness to methacholine in patients with impaired left ventricular function. N. Engl. J. Med. 320: 1317-1322.

Campbell BC, Sturani A, Reid JL (1985).
Evidence of parasympathetic activity of the angiotensin converting inhibitor captopril in normotensive man. Clin.Science 68: 49-56.

Campbell DJ (1985).
The site of angiotensin production. J. Hypertension 3: 199-209.

Captopril Collaborative Study Group (1982).
Does captopril cause renal damage in hypertensive patients ? Lancet i: 988-990.

Captopril Multicenter Research Group (1983).
A placebo controlled trial of captopril in refractory chronic congestive heart failure.
J.Am.Coll.cardiol. 2: 755-763.

Carbonell LF, Carretero OA, Stewart JM, Scicli AG (1988).
Effect of a kinin antagonist on the acute antihypertensive activity of enalaprilat in severe hypertension. Hypertension 11: 239-242.

Carroll JD, Hess OM, Krayenbuehl HP (1988).
Diastolic function during exercise induced ischaemia in man.
In: Grossman W, Lorell BH, eds. Diastolic Relaxation of the heart. 217-219. Boston.
Martinus Nijhoff Publishing Inc.

Chadda K, Goldstein S, Byington R, Curb JD (1986).
Effect of propranolol after acute myocardial infarction in patients with congestive heart failure. Circulation 73: 503-510.

Chakko S, Wokka D, Martinez H, De Marchena E, Futterman L, Kessler KM, Myerburg RJ (1991).
Clinical, radiographic and haemodynamic correlations in chronic congestive heart failure: conflicting results may lead to inappropriate care. Am.J.Med. 90: 353-359.

Chatterjee K, Rouleau J-L, Parmley WW (1982).

Haemodynamic and myocardial metabolic effects of captopril in chronic heart failure.
Br.Heart J. 47: 233-238.

Chiknas S. (1979)
Liquid chromatography assisted assay for ACE in serum. Clin.Chem. 25: 1259-1262.

Chen X, Pitt BR, Moalli R, Gillis CN (1984).
Correlation between lung and plasma angiotensin converting enzyme and the hypotensive effect of captopril in conscious rabbits. J.Pharm.Exp.Ther. 229: 649-653.

Chinese Cardiac Study Collaborative Group (1995).
Oral captopril versus placebo among 13634 patients with suspected acute myocardial infarction: interim report from the Chinese Cardiac Study (CCS-1).
Lancet 345: 686-687.

Cirillo VJ, Gomez HJ, Salonen J, et al (1988).
Lisinopril: dose-peak effect relationship in essential hypertension.
Br.J.Clin.Pharmacol. 25: 533-538.

Clarke KW, Gray D, Hampton JR (1994).
Evidence of inadequate investigation and treatment of patients with heart failure.
Br.Heart J. 71: 584-587.

Clausen J, Felsby M, Jorgensen FS, et al (1966).
Absence of prophylactic effect of propranolol in myocardial infarction. Lancet 2: 920-924.

Cleland JGF, Dargie HJ, Hodsman GP, et al (1984).
Captopril in heart failure. A double-blind controlled trial. Br.Heart J. 52: 530-535.

Cleland JGF, Dargie HJ, McAlpine H, Ball SG, Robertson JIS, Ford I (1985a).
Severe hypotension after first dose of enalapril in heart failure. Br.Med.J. 291: 1309-1312.

Cleland JGF, Dargie HJ, Ball SG, Gillen G, Hodsman GP, Morton JJ, et al (1985b).
Effects of enalapril in heart failure: a double blind study of effects on exercise performance, renal function, hormones, and metabolic state. Br.Heart J. 54: 305-312.

Cleland JGF, Dargie HJ. (1987)
Heart failure, renal function and angiotensin converting enzyme inhibitors.
Kidney Int. 31 (Suppl.20): S220-S228.

Cleland JGF, Dargie HJ, Robertson I, Robertson JIS, East BW (1987).
Total body electrolyte composition in patients with heart failure: a comparison with normal subjects and patients with untreated hypertension. Br. Heart J. 58: 230-238.

Cleland JGF, Oakley CM (1991).
Vascular tone in heart failure: the neuroendocrine-therapeutic interface.
Br.Heart J. 66: 264-267.

Cleland JGF, Erhardt L, Hall AS, Winter C, Ball SG (1993).

Validation of primary and secondary outcomes and classification of mode of death among patients with clinical evidence of heart failure: a report from the acute infarction ramipril efficacy (AIRE) study investigators. J.Cardiovasc.Pharmacol 22: S22-S27.

Cleland JGF, Erhardt L, Murray G, Hall AS, Ball SG (1997).
Effect of ramipril on morbidity and mode of death among survivors of acute myocardial infarction with clinical evidence of heart failure. Eur,Heart J. 18: 41-51.

Coats AJS, Adamopoulos S, Meyer TE, Conway J, Sleight P (1990).
Effects of physical training in chronic heart failure. Lancet 335: 63-66.

Cody RJ, Covit AB, Schaer GL, Laragh JH (1984).
Estimation of angiotensin II receptor activity in chronic congestive heart failure.
Am.Heart J. 108: 81-89.

Coghlan JP, Fie DTN, Scoggins BA, Tregear CW (1982).
Angiotensin production and metabolism in sheep. Clin. Exp. Pharm. Physiol. & (Suppl. 1); 21-29.

Cohen ML, Kurz KD (1982).
Angiotensin converting enzyme inhibition in tissue from spontaneously hypertensive rats after treatment with captopril or MK-421. J.Pharmacol.Exp.Ther. 220: 63-69.

Cohn, JN (1982).
Clinical definitions and studies.
In: Braunwald E, Mock MB, Watson J, Eds.Congestive Heart Failure.New York: Grune and Stratton 11-13.

Cohn JN, Levine TB, Olivari MT, et al (1984).
Plasma norepinephrine as a guide to prognosis in patients with chronic congestive heart failure. N.Engl.J.Med. 311: 819-823.

Cohn JN, Archibald DG, Ziesche S, et al (1986).
Effects of vasodilator therapy on mortality in chronic congestive heart failure. Results of a Veterans administration Cooperative Study. N.Eng.J.Med. 314: 1547-1552.

Cohn JN, Archibald DG, Francis GS, et al (1987).
Veterans Administration Cooperative Study of Vasodilator Therapy in Heart Failure: influence of prerandomisation variables on the reduction of mortality by treatment with hydralazine and isosorbide dinitrate. Circulation 75 (Suppl IV): 49-54.

Cohn JN, Johnson G, Ziesche S, et al (1991).
A comparison of enalapril with hydralazine-isosorbide dinitrate in the treatment of chronic congestive heart failure. N.Engl.J.Med. 325: 303-310.

Colucci WS, Ribeiro JP, Rocco MB, Quigg RJ, Creager MA, Marsh JD, et al (1989).

impaired chronotropic response to exercise in patients with congestive heart failure . Role of postsynaptic beta adrenergic desensitisation. Circulation 80: 314-323.

CONSENSUS Trial Study Group (1987).
Effects of enalapril on mortality in severe congestive heart failure: enalapril survival study. N.Engl.J.Med. 316: 1429-1435.

Cooper RA (1983).
Captopril-associated neutropenia: who is at risk ? Arch.Int.Med. 143: 659-660.

Cooper WD, Sheldon D, Brown D, Kimber GR, Isitt VL, Currie WJC (1987).
Post marketing surveillance of enalapril: experience in 11710 patients in general practice. J.Royal Coll. Gen. Pract. 37: 346-349.

Covit AB, Schaer GL, Sealey JE, Laragh JH, Cody RJ (1983).
Suppression of the renin-angiotensin system by intravenous digoxin in chronic congestive heart failure. Am.J.Med. 75: 445-447.

Cowley AJ, Skene AM. (1994).
Treatment of severe heart failure: quantity or quality of life. A trial of enoximone. Br.Heart J. 72: 226-230.

Creager MA, Halperin JL, Bernard DB, Faxon DP, Melidossian CD, Gavras H, Ryan TJ (1981).
Acute regional circulatory and renal hemodynamic effects of converting-enzyme inhibition in patients with congestive heart failure. Circulation 64: 483-489.

Crozier IG, Ikram H, Nicholls MG, Jans S (1989).
Global and regional hemodynamic effects of ramipril in congestive heart failure. J.Cardiovasc.Pharmacol. 14: 688-693.

Cushman DW, Cheung HS (1971).
Spectrophotometric assay and the properties of the angiotensin converting enzyme of rabbit lung. Biochem.Pharmacol. 20: 1637-1642.

Cushman DW, Wang FL, Fung WC, Harvey CM, DeForrest JM. (1989).
Differentiation of angiotensin converting (ACE) inhibitors by their selective inhibition of ACE at physiologically important target organs. Am.J.Hypertens. 2: 294-306.

Daemen MJAP, Lombardi DM, Bosman FT, Schwartz SM (1991).
Angiotensin II induces smooth muscle cell proliferation in the normal and injured rat arterial wall. Circ.Res. 68: 450-456.

Dall JLC. (1970).
Maintenance digoxin in elderly patients. Br.Med.J. 2: 705-706.

Danser AHJ, Koning MMG, Admiraal PJJ, Derkx FHM, Verdouw PD, Schalekamp MADH (1992).

Metabolism of angiotensin I by different tissues in the intact animal.
Am.J.Physiol. 263: H418-H428.

Danser AHJ, Koning MMG, Admiraal PJJ, Derkx FHM, Verdouw PD, Schalekamp MADH (1992).
Production of angiotensins I and II at tissue sites in intact pigs.Am.J.Physiol. 263: H429-H437.

Das Gupta P, Broadhurst P, Raftery EB, Lahiri A. (1990).
Vakue of carvedilol in congestive heart failure secondary to coronary artery disease.
Am.J. Cardiol. 66: 1118-1123.

Davies RH, Sheridan DJ (1993).
The treatment of heart failure- what next ? Br.J.Clin.Pharmac. 35: 557-563.

Davies SW, Emery TM, Watling MIL, Wannamathee G, Lipkin DP. (1991).
A critical threshold of exercise capacity in the ventilatory response to exercise in heart failure. Br.Heart J. 65: 179-183.

Davies SW, Greig CA, Jordan SL, Grieve DW, Lipkin DP (1992).
Short-stepping gait in severe heart failure. Br.Heart J. 55: 469-472.

Davies SW (1993).
Exercise physiology in heart failure. ACE Reports 103: 1-6.

Davis JO, Freeman RH (1976).
Mechanisms regulating renin release. Phys. Rev. 56: 1-56.

de Vries RJM, Dunselman PHJM. (1995).
The potential role of calcium antagonists in the management of congestive heart failure: initial experience with lacidipine. J.Cardiovasc.Pharmacol. 25(Suppl. 3): S33-S39.

de Silva PE, Husain A, Smeby RR, Khairallah PA (1988).
Measurement of immunoreactive angiotensin peptides in rat tissues: some pitfalls in angiotensin II analysis. Anal.Biochem. 174: 80-87.

Deck CC, Gabella MA, Raya TE (1996).
Relative contributions of angiotensin II, bradykinin, and prostaglandins to the renal effects of converting enzyme inhibition in rats after chronic myocardial infarction. J.Cardiovasc.Pharmacol. 28: 167-174.

Dei Cas L, Metra M, Nodari S, Visoli O (1989).
Efficacy of ibopamine treatment in patients with advanced heart failure: purpose of a new therapeutic scheme with multiple daily administrations.
J.Cardiovasc.Pharmacol. 14 (Suppl 8): S111-S117.

Demakis JG, Proskey A, Rahimtoola SH (1974).
The natural course of alcoholic cardiomyopathy. Ann.Intern.Med. 80: 293-297.

Derkx FHL, Tan-Tjiong HL, Man In't Veld AJ, Schalekamp MADH. (1979)
Activation of inactive plasma renin by plasma and and tissue kallikreins.Clin.Sci. 57: 351-357.

Devlin A (1992).
Development of an analytical method for the specific assay of angiotensin II in human plasma by the combination of h.p.l.c. with r.i.a. Brit.J.Clin.Pharmac. 35: 457P.

DiBianco R, Shabetai R, Kostuk W, Moran J, Schlant RC, Wright R, for the Milrinone Multicenter Trial Group (1989).
A comparison of oral milrinone, digoxin, and their combination in the treatment of patients with chronic heart failure. N.Engl.J.Med. 320: 677-683.

Di Carlo L, Chatterjee K, Parmley WW, et al (1983).
Enalapril: a new angiotensin converting enzyme inhibitor in chronic heart failure: acute and chronic haemodynamic evaluations. J.Am.Coll.Cardiol. 2: 865-871.

Dies F, Knell MJ, Whitlow P, Liang CS, Goldenberg I, Applefield MM, Gilbert EM. (1984).
Intermittent dobutamine in ambulatory outpatients with chronic cardiac failure. Circulation 74 (Suppl. II): II-39.

Dougherty AN, Naccarelli GV, Gray EL, Hichs CH, Goldstein RA (1984).
Congestive heart failure with normal systolic function. Am.J.Cardiol. 54: 778-782.

Donnelly R, Meredith PA, Elliott HL, Reid JL (1990).
Kinetic-dynamic relations and individual responses to enalapril. Hypertension 15: 301-309.

Dosemeci A, Dhallan RS, Cohen NM, et al (1988).
Phorbol ester increases calcium current and simulates the effects of angiotensin II on cultured neonatal rat haert myocytes. Circ. Res 62: 347-357.

Drexler H, Riede U, Munzel T, Konig H, Funke E, JUST H (1992).
Alterations of skeletal muscle in chronic heart failure. Circulation 85: 1752-1759.

Dunselman PHJM, Kuntze CEE, Van Bruggen A, et al (1989).
Efficacy of felodipine in congestive heart failure. Eur.Heart J.10: 354-364.

Dupuis J, Lalonde G, Lemieux R, Rouleau JL (1990).
Tolerance to intravenous nitroglycerin in patients with congestive heart failure: role of increased intravascular volume, neurohumoral activation and lack of prevention with N-acetyl cysteine. J.Am.Coll.Cardiol. 16: 923-931.

Dzau VJ, Colucci WS, Hollenberg NK, Williams GH (1981).
Relation of the renin-angiotensin-aldosterone system to clinical state in congestive heart failure. Circulation 63: 645-651.

Dzau VJ, Kopelman RI, Barger AC, Haber C (1980).
Renin specific antibody for the study of cardiovascular homeostasis.
Science 207: 1091-1093.

Dzau VJ, Ingelfinger J, Pratt RE, Ellison KE (1986).
Identification of renin and angiotensinogen messemger RNA sequences in mouse and rat brains. Hypertension 8: 544-548.

Dzau VJ (1987).
Implications of local angiotensin production in cardiovascular physiology and pharmacology. Am.J.Cardiol. 59: 59A-65A.

Dzau VJ (1988).
Vascular renin angiotensin system in hypertension. New insights into the mechanism of action of angiotensin converting enzyme inhibitors. Am.J.Med. 84: 4-8.

Dzau VJ (1988).
Circulating versus local renin-angiotensin system in cardiovascular homeostasis.
Circulation 77 (Suppl. 1): 4-9.

Dzau VJ, Safar ME (1988).
Large conduit arteries in hypertension : role of the vascular renin angiotensin system.
Circulation 77: 947-953.

Dzau VJ, Horiuchi M (1996).
Differential expression of angiotensin receptor subtypes in the myocardium: a hypothesis.
Eur.Heart J.17: 978-980.

Editorial (1989).
Clinical signs in heart failure. Lancet 334:309-310.

Edwards RM (1983).
Segmental effects of norepinephrine and angiotensin II on isolated renal microvessels.
Am.J.Physiol. 244: F526-F534.

Elkayam U, Roth A, Mehra A, et al (1991).
Randomised study to evaluate the relation between oral isosorbide dinitrate dosing interval and the development of early tolerance to its effect on left ventricular filling pressure in patients with chronic heart failure. Circulation 84: 2040-2048.

Elliott HL, Macdonald NJ, Meredith PA, Reid JL (1992).
Dose response and pharmacokinetics for the angiotensin converting enzyme inhibitor quinapril. Clin. Pharmacol. Ther. 51:260-265.

Erdos EG (1990).
Angiotensin I converting enzyme and the changes in our concepts through the years.
Hypertension 16: 363-370.

Eriksson H, Svardsudd K, LArsson B, et al (1989).
Risk factors for heart failure in the general population. The study of men born in 1913.
Eur. Heart J. 10: 647-656.

Eriksson H, Wilhelmsen L, Cardahl K, Svardsudd K (1991).
Epidemiology and prognosis of heart failure. Zeitschr.Kardiol. 80 (Suppl 8): 1-6.

Ertl G, Kloner RA, Alexander RW, Braunwald E (1982).
Limitation of experimental infarct size by an angiotensin converting enzyme inhibitor.
Circulation 65: 40-48.

Fabris B, Jackson B, Mendelsohn FAO, Johnston CI (1989).
Angiotensin converting enzyme in the rat heart: studies of its inhibition in vitro and ex
vivo. Clin.Exp.Pharmacol.Physiol. 16: 309-313.

Fabris B, Jackson B, Kohzuki M, Perich R, Johnston CI (1990).
Increased cardiac angiotensin converting enzyme in rats with chronic heart failure.
Clin.Exp.Pharmacol.Physiol. 17: 309-314.

Farrow PR, Wilkinson R (1979).
Reversible renal failure during treatment with captopril. Br.Med.J. 1: 1680.

Feldman AM, Baughman KL, Lee WK, et al. (1991).
Usefulness of OPC-8212, a quinolone derivative, for chronic congestive heart failure in
patients with ischaemic heart disease or idiopathic dilated cardiomyopathy.
Am.J.Cardiol. 68: 1203-1210.

Feldman AM, Bristow MR, Parmley WW, Carson PE, et al. (1993).
Effects of vesnarinone on morbidity and mortality in patients with heart failure.
N.Engl.J.Med 329: 149-155.

Feldman MD, Copelas L, Gwathmey JK, et al (1987).
Deficient production of cyclic AMP: pharmacologic evidence of an important cause of
contractile dysfunction in patients with end-stage heart failure.Circulation 75: 331-339.

Ferry JJ, Horvath AM, Sedman AJ, Latts JR, Colburn WA (1987).
Influence of food on the pharmacokinetics of quinapril and its diacid metabolite CI-928. J.
Clin. Pharmacol. 27:397-399.

Fitzpatrick MA, Nicholls MG, Ikram H, Espiner EA (1985).
Stability and inter-relationships of hormone, haemodynamic and electrolyte levels in heart
failure in man. Clin.Exp.Pharmac.Physiol. 12: 145-154.

Ferguson DW, et al (1990).
Clinical and haemodynamic correlates of sympathetic nerve activity in normal humans and
patients with heart failure: evidence from direct microneurographic recordings.
J.Am.Coll.Cardiol. 16: 1125-1134.

Feuerstein GZ, Ruffolo RR (1995).
Carvedilol, a novel multiple action antihypertensive agent with antioxidant activity and the potential for myocardial and vascular protection. Eur. Heart J. 16 (Suppl F): 38-42.

Fink LI, Wilson JR, Ferraro N (1986).
Exercise ventilation and pulmonary artery wedge pressure in chronic stable congestive heart failure. Am.J.Cardiol. 57: 249-253.

Fitzpatrick MA, Rademaker MT, Frampton CM, Charles CJ, Yandle TG, Espiner EA, Ikram H (1990).
Haemodynamic and hormonal effects of renin inhibition in ovine heart failure. Am.J.Physiol. 258: H1625-1631.

Fleg JL, Rothfeld B, Gottlieb SH (1991).
Effect of maintenance digoxin therapy on aerobic performance and exercise left ventricular function in mild to moderate heart failure due to coronary artery disease: a randomised, placebo-controlled crossover trial. J.Am.Coll.Cardiol. 17: 743-751.

Fonrose HA, Ahlbaum N, Bugatch E, Cohen M, Genovese C, Kelly J. (1974)
The efficacy of digitalis withdrawal in an institutional aged population.J.Am.Geritric.Soc 22: 208-211.

Foresti V, Pepe R, Parisio E, de Fillipi G, Scotari N, Frigerio C (1989).
Angiotensin converting enzyme as a possible marker for lung toxicity in amiodarone treated patients. Int.J.clin.Pharm.Res. 10: 261-267.

Foult J-M, Tavolaro O, Antony I, Nitenberg A (1988).
Direct myocardial and coronary effects of enalaprilat in patients with dilated cardiomyopathy: assessment by a bilateral intracoronary infusion technique. Circulation 77: 337-344.

Fowler MB, Laser JA, Hopkins GL, Minobe W , Bristow MR. (1986).
Assesment of the beta adrenergic pathway in in the intact failing human heart: progressive receptor down-regulation and subsensitivity to agonist response. Circulation 74: 1290-1302.

Francis R, Brown A, Kler L, D'Amore F, Nussberger J, Waeber B, Brunner H (1987).
Pharmacokinetics of the converting enzyme inhibitor cilazapril in normal volunteers and the relationship to enzyme inhibition: development of a mathematical model. J.Cardiovasc.Pharmacol. 9: 32-38.

Franciosa JA, Park M, Levine TB (1981).
Lack of correlation between exercise capacity and indexes of resting left ventricular performance in heart failure. Am.J.Cardiol. 47: 33-39.

Franciosa JA, Baker JB, Seth L (1985).
Pulmonary versus systemic haemodynamics in determining exercise capacity of patients with chronic left ventricular failure. Am.Heart.J. 110: 807-813.

Franciosa JA, Wilen M, Ziesche S, Cohn JN (1983).
Survival in men with severe chronic left ventricular failure due to either coronary artery disease or idiopathic dilated cardiomyopathy. Am.J.Cardiol. 51: 831-836.

Franciosa JA, Schwartz DE (1989).
Acute haemodynamic effects of norepinephrine inhibition in patients with severe congestive heart failure. J.Am.Coll.Cardiol. 14: 624-630.

Francis GS (1987).
Haemodynamic and neurohumoral responses to dynamic exercise: normal subjects versus patients with heart disease. Circulation 76: VI 11-VI 17.

Francis R, Brown A, Kler L, D'amore F, Nussberger J, Waeber B, Brunner H (1987).
Pharmacokinetics of the converting enzyme inhibitor cilazapril in normal volunteers and the relationship to enzyme inhibition: development of a mathematical model. J.Cardiovasc.Pharmacol. 9: 32-38.

Francis GS, Benedict C, Johnstone DE, Kirlin PC, Nicklas J, Liang C, Kubo SH, Rudin-Toretsky E, Yusuf S (1990).
Comparison of neuroendocrine activation in patients with left ventricular dysfunction with and without congestive heart failure. A substudy of the studies of left ventricular dysfunction (SOLVD). Circulation 82: 1724-1729.

Francis GS, Cohn JN, Johnson G, et al. (1993).
Plasma norepinephrine, plasma renin activity and congestive heart failure. Relations to survival and the effects of therapy in VHeFT-II. Circulation 87 (Suppl. VI): VI40-VI48.

Franklin SS, Smith RD (1985).
Comparison of effects of enalapril plus hydrochlorothiazide versus standard triple therapy on renal in renovascular hypertension. Am.J.Med. 79 (3C): 14-23.

Freeman RH, Davis JO, Williams GM, DeForrest JM, Seymaour AA, Rowe BP (1979).
Effects of the oral converting enzyme inhibitor, SQ 14225, in a model of low cardiac output in dogs. Circ.Res. 45: 540-545.

Frohlich ED, Cooper RA, Lewis EJ (1984).
Review of the overall experience of captopril in hypertension. Arch.Int.Med. 144: 1441-1444.

Fujino K, Sperelakis N, Solaro RJ. (1988).
Sensitisation of dog and guinea-pig myofilaments to Ca++ activation and the inotropic effect of pimobendan: comparison with milrinone. Circ.Res. 63: 911-922.

Furburg CD, Yusuf S. (1985).
Effects of vasodilators on survival in chronic congestive heart failure. Am. J.Cardiol. 55: 1110-1113.

Fyhrquist F, Gronhagen-Riska C, Hortling L, Hichens M (1982).
Induction of angiotensin I converting enzyme in rat lung with captopril: effect of adrenalectomy. Am.J.Cardiol. 49: 1508-1510.

Gaillard CA, Koomans HA, Rabelink TJ, Boer P, Dorhout Mees EJ (1989).
Opposite effects of enalapril and nitrendipine on natriuretic response to atrial natriuretic factor. Hypertension 13: 173-180.

Geisterfer A, Peach MJ, Owens GK (1988).
Angiotensin II induces hypertrophy, not hyperplasia of cultured rat aortic smooth muscle cells. Circ.Res. 62: 749-756.

Geyskes GG, Oei HY, Puylaert CBAJ, Dorhout EJ (1987).
Renovascular hypertension identified by captopril-induced changes in the renogram. Hypertension 9: 451-458.

Ghali JK, Cooper R, Ford E (1990).
Trends in hospitalisation rates for heart failure in the United States 1973-1986. Arch.Inter.Med. 150: 769-773.

Ghali JK, Kadakia S, Cooper RS, Liao Y. (1991)
Bedside diagnosis of preserved versus impaired left ventricular systolic function in heart failure. Amer.J.Cardiol. 67: 1002-1006.

Gilchrist NL, Richards AM, March R, Nicholls MG (1989).
Effects of sulindac on angiotensin converting enzyme inhibitor-induced cough: randomised, placebo-controlled, double-blind crossover study. J.Hum.Hypertens. 3: 451-455.

Goldblatt H, Lynch J, Hanzal RF, Summerville WW (1934).
Studies on experimental hypertension. I. The production of persistent elevation of systolic blood pressure by means of renal ischaemia. J.Exp.Med. 59: 347-380.

Goldsmith SR, Francis GS, Cowley AL, Levine TB, Cohn JN (1983).
Increased arginine vasopressin levels in patients with congestive heart failure. J.Am.Coll.Cardiol. 1: 1385-1390.

Gorski TP, Campbell DJ (1991).
Angiotensin-converting enzyme determination in plasma during therapy with converting enzyme inhibitor: two methods compared. Clin. Chem. 37: 1390-1393.

Griffin S, Brown W, Macpherson F, McGrath J, Mulvany MJ, Lever AF (1991).
Angiotensin II causes vascular hypertrophy in part by a non-pressor mechanism. Hypertension 17: 626-635.

Grim CE, Weinberger MH, Higgins JT, Kramer NJ (1977).
Diagnosis of secondary forms of hypertension: a comprehensive protocol. J.Am.Med.Assoc. 237: 1331.

Grundmeijer HGLM, Meeter KA, Hoes AW, Mosterd A (1996).
Heart failure: meaning of symptoms and clinical findings for the general practitioner.
Huisarts en Wetenschap 39: 3-11.

Gundersen T, Swedberg K, Amtorp O, Remes J, Nilsson B (1994).
Absence of effect on exercise capacity of 12 weeks treatment with ramipril in patients with moderated congestive heart failure. Eur. Heart J. 15: 1659-1665.

Gupta S, Why HJF, Jewitt DE, Monaghan MJ (1995).
Changing trends in an echocardiographic service: a five year retrospective audit.
(Abstract) Br.Heart J. 73 (Suppl 2):41.

Graves EJ (1992).
National hospital discharge survey. Annual summary 1990, National Center for Health Statistics. Vital Health Stat. 13: 1-62.

Grossman W (1990).
Diastolic dysfunction and congestive heart failure. Circulation 81: 1-7

Halstenson CE, Opsahl JA, Rachael K, Olson SC, Horvath AM, Abraham PA, Posvar EL (1992).
The pharmacokinetics of quinapril and its active metabolite, quinaprilat, in patients with various degrees of renal function. J.Clin.Pharmacol. 32: 344- 350.

Hansen DE, Craig CS, Hondeghem LM (1990).
Stretch-induced arrhythmias in the isolated canine heart. Circulation 81: 1094-1105.

Harlan WR, Obermann A, Grimm R, Rosati RA (1977).
Chronic congestive heart failure in coronary artery disease: clinical criteria.
Ann.Intern.Med. 86: 133-138.

Harrigan JR, Hughes DM, Meredith PA, Reid JL.
Characterisation of the effects of prodrug concentration on the in vitro potency of metabolites of five ACE inhibitors.(Abs) Eur.J.Clin.Pharmacol. 1989 36 (Suppl.): A186.

Hart W, McMurray J (1993).
The cost effectiveness of heart failure in the treatment of heart failure. Eur.Heart J. 14: Suppl. 14-17.

Hart W, Rhodes G, McMurray J (1993).
The cost effectiveness of heart failure in the treatment of chronic heart failure.
Br.J.Med.Econ 6: 91-98.

Hasenfuss G, Holubarsch C, Heiss HW, Rattert B, Just HJ. (1989).
Influence of the calcium sensitiser UDCG-115 on haemodynamics and myocardial energetics in patients with idiopathic dilated cardiomyopathy. Comparison with nitroprusside. In: Just HJ, Holubarsch C, Scholz H. Eds. Inotropic Stimulation and

Myocardial Energetics. Springer-Verlag, New York 225-233.

Harrap SB, Van der Merwe WM, Griffin SA, MacPherson F, Lever AF (1990).
Brief angiotensin converting enzyme inhibitor in young spontaneously hypertensive rats reduces blood pressure long-term.Hypertension 16: 603-617.

Harrap SB (1991).
Angiotensin converting enzyme inhibitors, regional vascular haemodynamics, and the development and prevention of experimaental genetic hypertension.
Am.J.Hypertens. 4: 2125-2165.

Hasford J, Bussman WD, Delius W, et al (1991).
First dose hypotension with enalapril and prazosin in congestive heart failure.
Int.J.Cardiol. 31: 287-294.

Hay JW, Wittels EH, Gotto Jr AM (1991).
An economic evaluation of lovastatin for cholesterol lowering and coronary artery disease reduction.Am.J.cardiol. 67: 789-796.

Hedner T, Samuelson O, Lunde H, Lindholm L, Andren L, Eilholm BE (1992).
Angio-oedema in relation to treatment with angiotensin converting enzyme inhibitors.
Brit.Med.J. 304: 941-946.

Higgens CB, Vatner SF, Sckberg DL, Braunwald E (1972).
Alterations in the baroreceptor reflex in conscious dogs with heart failure.
J.Clin.Invst. 51: 715-724.

Higginbotham MB, Morris KG, Conn EH, Coleman RE, Cobb FR (1983).
Determinants of variable exercise performance among patients with severe left ventricular dysfunction. Am.J.Cardiol. 51: 52-60.

Hilgers KF, Peters J, Veelken R, Sommer M, Rupprecht G, Ganten D, et al (1992).
Increased vascular angiotensin formation in female rats harboring the mouse Ren-2 gene.
Hypertension 19: 687-691.

Hirakata H, Fouad-Tarazi PM, Bumpus FM, Khosla M, Healy B, Husain A et al (1990).
Angiotensin and the failing heart. Enhanced positive inotropic response to angiotensin I in cardiomyopathic hamster heart in the presence of captopril. Circ.Res. 66: 891-899.

Ho KK, Pinsky JL, Kannel WB, Levy D (1993a).
The epidemiology of heart failure: the Framingham Study. J.Am.Coll.Cardiol. 22: 6A-13A.

Ho KK, Anderson KM, Kannel WB, Grossman W, Levy D (1993b).
Survival after the onset of congestive heart failure in Framingham Heart Study subjects.
Circulation 88: 107-115.

Hodsman GP, Isles CG, Murray GD, Usherwood TP, Webb DJ, Robertson JIS (1983).
Factors related to first dose hypotensive effects of captopril: prediction and treatment.

Br.Med.J. 286: 832-834.

Hodsman GF, Zabludowski JR, Zoccali C et al (1984).
Enalapril (MK 421) and its lysine analogue (MK 521); a comparison of acute and chronic effects on blood pressure, renin-angiotensin system and sodium in normal man. Br.J.Clin.Pharmacol. 17: 233-241.

Hollenberg NK (1983).
Medical therapy of renovascular hypertension: efficacy and safety of captopril in 269 patients. Cardiovasc.Rev.Rep. 4: 852-878.

Hollenberg NK (1993).
A buoyant view of the value of angiotensin converting enzyme inhibition in renovascular disease. In:The Renin Angiotensin System: pp 90.1-90.8. J.I.S. Robertson, M.G.Nicholls, Editors. Gower Medical Publishing, London.

Holt P, Naim J. Sowton E (1986).
The acute haemodynamic effects of quinapril, a new non- sulfhydryl angiotensin converting enzyme inhibitor, in patients with severe congestive cardiac failure. Eur.J.Clin.Pharmacol. 31:9-14.

Hood WB, Youngblood M, Ghali JK, et al. (1991).
Initial blood pressure response to enalapril in hospitalised patients (Studies of Left Ventricular Dysfunction [SOLVD]). Am.J.Cardiol. 68: 1465-1468.

Horton R, Biglieri EG (1962).
Effect of aldosterone on the metabolism of magnesium. J.Clin.Endocrinol.Metab. 22: 1187-1192.

Horvath AM, Pilon D, Caille G, Colburn WA (1990).
Multiple-dose propranolol administration does not influence the single dose pharmacokinetics of quinapril and its active metabolite (quinaprilat). Biopharmaceutics and Drug Disposition 11:191-196.

Hostetter TH, Rennke HG, Brenner BM (1982).
The case for intra-renal hypertension in the initiation and progression of diabetic and other glomerulopathies. Am.J.Med. 72: 375-380.

Houghton AR, Cowley AJ (1996).
The use of angiotensin converting enzyme inhibitors in heart failure by General Practitioners: results of a postal survey. (Abstract). Eur.Heart J. 17: (Suppl.): P410

Hull SM, Mackintosh A.(1977)
Discontinuation of maintenance digoxin therapy in general practice.Lancet 2: 1054- 1055.

Ideishi M, Sasaguri M, Ikeda M, Arakawa K (1990).
Substrate-dependent angiotensin II formation in the peripheral circulation. Life Sci. 46: 335-341.

Ikram H, Maslowski AH, Smith BL, Nicholls MG (1981).
The haemodynamic, histopathological and hormonal features of alcoholic cardiac beri-beri.
Q.J.Med. 50: 359-375.

Inagami T, Murakami T, Higuchi K, Nakajo S (1991).
Roles of renal and vascular renin in spontaneous hypertension and switching of the
mechanism upon nephrectomy. Am. J. Hypertension 4 (Suppl.): 15-22.

Inagami T (1993).
Renin in the testis and epidydimis. In: Robertson JIS, Nicholls MG, Eds.. The renin-
angiotensin system, Volume 1 London, Gower : 47.1-47.5.

Ingelfinger JR, Pratt RE, Ellison K, Dzau VJ (1986).
Sodium regulayion of angiotensinogen mRNA expression in rat kidney cortex and
medulla. J.Clin.Invest. 78: 1311-1315

Ingelfinger JR, Pratt RE, Dzau VJ (1988).
Regulation of extra-renal renin during ontogeny. Endocrinology 122: 782-786.

Ingelfinger JR, Zou WM, Fon EA, Ellison KE, Dzau VJ (1990).
In situ hybridisation evidence for angiotensinogen messenger RNA in the rat proximal
tubule. J.Clin.Invest. 85: 417-423.

Inman WHW, Rawson NSB, Wilton LV, Pearce GL, Speirs CJ (1988).
Post marketing surveillance of enalapril. I Results of prescription event monitoring.
Brit.Med.J. 297: 826-829.

Insua A, Ribstein J, Mimran A (1988).
Comparative effect of captopril and nifedipine in normotensive patients with incipient
diabetic nephropathy. Postgrad.Med.J. 64 (Suppl. 3): 59-62.

ISIS-1. First Randomised Study Of Infarct Survival Collaborative Group (1986).
Randomised trial of intravenous atenolol among 16027 cases of suspected acute
myocardial infarction. Lancet 1: 57-66

ISIS-4 (Fourth International Study of Infarct Survival) Collaborative Group.
ISIS-4: a randomised factorial trial assessing early oral captopril, oral mononitrate, and
intravenous magnesium sulphate in 58050 patients with suspected acute myocardial
infarction. N.Engl.J.Med. 327: 669-677.

Jackson B, Cubela R, Johnston CI (1986).
Angiotensin converting enzyme (ACE) characterisation by 125I-MK351A binding studies
of plasma and tissue ACE during variation of salt status in the rat.
J.Hypertension 4: 759-765.

Jackson B, Cubela R, Johnston CI (1988).
Effects of perindopril on angiotensin converting enzyme in tissues of the rat.

J.Hypertension 6 (Suppl. 3): 551-554.

Jaeschke RJ, Owman AD, Guyatt GH. (1990).
To what extent do congestive heart failure patients in sinus rhythm benefit from digoxin therapy ? Am.J. Med. 1990 88: 279-286.

Johnson JA, Davis JO (1973).
Angiotensin II: important role in the maintenance of arterial blood pressure.
Science 179: 906-907.

Johnston CI, Fabris B, Yamada H, Mendelsohn FAO, Cubela R, Sivell D, Jackson B (1989).
Comparative studies of tissue inhibition by angiotensin converting enzyme inhibitors.
J.Hypertens. 7 (Suppl.) S11-S16.

Johnston CI.
Angiotensin converting enzyme inhibitors. IN: Handbook of Hypertension Vol II: Antihypertensive drugs. Doyle AE, Editor pp 301-326. Elsevier, Amsterdam.

Kalil RSN, Katz SA, Keane WF (1993).
Angiotensin converting enzyme inhibitors in diabetes mellitus.
IN: The Renin Angiotensin System. pp 92.1-92.20 J.I.S. Robertson, M.G.Nicholls, Editors. Gower Medical Publishing, London.

Kannel WB (1987).
Epidemiology and prevention of heart failure: Framingham study insights. Eur.Heart J. 8 (Suppl F): 23-26.

Kannel WB, Plehn JF, Cupples LA (1988).
Cardiac failure and sudden death in the Framingham Study. Am. Heart J. 115: 869-875.

Kannel WB (1989).
Epidemiological aspects of heart failure. Cardiol.Clin. 7: 1-9.

Kannel WB, Belanger AJ (1991).
Epidemiology of heart failure. Am.Heart J. 121: 951-957.

Kassis A, Amtorp O. (1990).
Long-term clinical, haemodynamic, angiographic and neurohumoral response to vasodilation with felodipine in patients with chronic congestive heart failure.
J.Cardiovasc.Pharmacol. 15: 347-352.

Kannel WB, Ho K, Thom T (1994).
Changing epidemiological features of cardiac failure. Br.Heart J. 72: S3-S9.

Kaufman MP, Baker DG, Coleridge HM, Coleridge JCG. (1980).
Stimulation by bradykinin and afferent vagal C-fibres with chemosensitive endings in the heart and aorta of the dog. Circ.Res. 46: 476-484.

Katz SD, Kubo SH, Jessup M, et al. (1996).
A multicenter, randomised, double-blind trial of pimobendan, a new cardiotonic and vasodilator agent, in patients with severe congestive heart failure.
Am.Heart J. 123: 95-103.

Kayanakis JG (1989).
The syndrome of "muscle deconditioning" in chronic cardiac insufficiency.
Arch.Mal.Coeur. 82: 1455-1485.

Kaye DM, Lambert GW, Lefkovits J, Morris M, Jennings G, Esler MD (1994).
Neurochemical evidence of cardiac sympathetic activation and increased central nervous system norepinephrine turnover in severe congestive heart failure.
J.Am.Coll.Cardiol. 23: 570-578.

Kelley J (1988).
Lavage angiotensin converting enzyme as a marker of lung injury.
Am.Rev.Resp.Dis. 137: 531-534.

Kelly P, Macauley-Hunter E, Lowry P, Perrins EJ (1995).
Novel prediction of heart failure by plasma concentration of various atrial natriuretic peptides.(Abstract). Eur.Heart J. 16 (Suppl.): P898 .

Kelso EJ, Mcdermott BJ, Silke B. (1995).
Actions of the novel vasodilator, flosequinan, in isolated ventricular cardiomyocytes.
J.Cardiovasc.Pharmacol. 25: 376-386.

Kemp GJ, Thompson CH, Stratton JR, Brunotte F, Conway M, Adamopoulos S, Arnolda L, et al (1996).
Abnormalities in exercising skeletal muscle in congestive heart failure can be explained in terms of decreased mitochondrial ATP synthesis, reduced metabolic efficiency and increased glycogenolysis. Heart 76: 35-41.

Ketley D, Woods KL. (1995).
Differences in the use of β-blocking drugs for secondary prevention after acute myocardial infarction in 11 European countries.(Abstract). Eur. Heart J. 16 (Suppl.): P 2832.

Kinoshita A, Urata H, Bumpus M, Husain A (1991).
Multiple determinants for the high substrate specificity of an angiotensin II forming chymase from the human heart. J.Biolog.Chem. 266: 19192-19197.

Kober L, Torp-Pedersen C, Carlsen JE, et al. (1995).
A clinical trial of the angiotensin converting enzyme inhibitor trandolapril in patients with left ventricular dysfunction after myocardial infarction.
N.Engl.J.Med. 333: 1670-1676.

Kohzuki H, Johnston C, Yeen Chai S, Jackson B, Perich R, Paxton D, Mendelsohn FAO.
Measurement of angiotensin enzyme induction and inhibition using quantitive in vivo

autoradiography: tissue selective induction after chronic lisinopril treatment.
J.Hypertens. 1991 9: 579-587.

Koilpillai C, Quinones MA, Greenberg B, et al (1996).
Relation of ventricular size and function to heart failure status and ventricular dysrhythmia
in patients with severe left ventricular dysfunction. Am.J.Cardiol. 77: 606-611.

Konstam MA et al, (1991).
Effects of enalapril on the progression of left ventricular dysfunction in heart failure.
Circulation 84 (Suppl II): 310A.

Koopmanschap MA, van Roijen L, Bonneux L (1992).
Costs of diseases in the Netherlands.
Report of the Department of Public Health and Social Medicine and the Institute for
Medical Technology Assessment. Erasmus University, Rotterdam.

Krum H, Sackner-Bernstein J, Goldsmith RL, et al. (1995).
Double-blind, placebo controlled study of the long term efficacy of carvedilol in patients
with severe chronic heart failure. Circulation 92: 1499-1506.

Kubo SH, Gollub S, Bourge, et al. (1992).
Beneficial effects of pimobendan on exercise tolerance and quality of life in patients with
heart failure. Results of a multicenter trial. Circulation 85: 942-949.

Kremer D, Lindop G, Brown WCB, Morton JJ, Robertson JJ (1981).
Angiotensin-induced myocardial necrosis and renal failure in the rabbit. distribution of
lesions and severity in relation to plasma angiotensin II comcentration and arterial
pressure. Cardiovasc.Res. 15: 43-46.

Kroc Collaborative Study Group (1984).
Blood glucose control and the evolution of diabetic retinopathy and albuminuria. A
preliminary multicenter trial. N.Engl.J.Med. 311: 365-372.

LaBarre Tr, O'Connell JB, Gunar RM.
Captopril therapy for severe CHF: hypotensive response in presence of markedly elevated
PRA. Am.Heart J. 1982 103: 308-309.

Lantz P. Enghoff E, Hedlund A, Hagg A.
Haemodynamic results of perorally administered converting inhibitors in congestive heart
failure. Scand.J.Urol.Nephrol. 1984 79 (Suppl): 129-132.

Landahl S, Svanborg A, Astrand K (1984).
Heart volume and the prevalence of certain common cardiovascular disorders at 70 and 75
years of age. Eur.Heart J. 5: 326-331.

Lathrop DA, Varro A, Schwartz (1989).
Rate-dependent electrophysiological effects of OPC-8212: comparison to sotalol.
Eur.J.Pharmacol. 164: 487-496.

Lavine SJ, Arends D (1989).
Importance of left ventricular filling pressure on diastolic filling in idiopathic dilated cardiomyopathy. Am. J. Cardiol. 64: 61-65.

Le Jemtel Th, Maskin CS, Lucido D, Chadwick BJ (1986).
Failure to augment maximal limb blood flow in response one-leg versus two-leg exercise in patients with severe heart failure. Circulation 74: 245-251.

Lee WH, Packer M (1986).
Prognostic importance of serum sodium concentration and its modification by converting enzyme inhibition in patients with severe chronic heart failure. Circulation 73: 257-267.

Lee ME, Miller WL, Edwards BS, Burnett JC (1989).
Role of endogenous atrial natriuretic factor in acute congestive heart failure.
J.Clin.Invest. 84: 1962-1966.

Lees KR, Kelman AW, Reid JL, Whiting B (1989).
Pharmacokinetics of an ACE inhibitor S9780 in man: evidence of tissue binding.
J.Pharmacokinet.Biopharm. 17: 529-550.

Leimbach WN, Wallin GB, Victor RG, Aylward PE (1986).
Direct evidence from intraneural recordings for increased sympathetic outflow in patients with heart failure. Circulation 73: 913-919.

Lele SS, MacFarlane D, Morrison S, Thomson H, Khafagi F, Frenneaux M (1996).
Determinants of exercise capacity in patients with coronary artery disease and mild to moderate systolic dysfunction. Eur.Heart J. 17: 204-212.

Leutscher JA, Kraemer FB (1988).
Microalbuminuria and increased plasma protein. Prevalence in diabetics followed up for four years. Arch.Int.Med. 148: 937-941.

Levens NR, Peach MJ, Carey RM (1981).
Role of the intrarenal renin-angiotensin system in the control of renal function.
Circ.Res. 48: 157-167.

Lindpainter K, Jin M, Niedermaier N, Wilhelm MJ, Ganten D (1990).
Cardiac angiotensinogen and its local activation in the isolated, perfused beating heart.
Circ. Res. 67: 564-573.

Lindsay DC, Lovegrove CA, Dunn MJ, Bennett JG, Pepper JR, Yacoub MH, Poole-Wilson PA (1996).
Histological abnormalities of muscle from limb, thorax and diaphragm in chronic heart failure. Eur.Heart J. 17: 1239-1250.

Lipkin DP, Jones DA, Round JM, Poole-Wilson PA (1988).
Abnormalities of skeletal muscle in patients with chronic heart failure.

Int.J.Cardiol. 18: 187-195.

Linz W, Scholkens BA, Han YI (1986).
Beneficial effects of the converting enzyme inhibitor ramipril in ischaemic rat hearts.
J.Cardiovasc.Pharmacol. 8 (Suppl 10): 591-599.

Linz W, Scholkens BA (1992).
A specific B_2-bradykinin receptor antagonist HOE 140 abolishes the antihypertrophic effect of ramipril. Br.J.Pharmacol 105: 771-772.

Lyons D, Webster J, Benjamin N (1997).
Effect of enalapril and quinapril on forearm vascular ACE in man.
Eur.J.Clin.Pharmacol. 51: 373-378.

MacFadyen RJ, Lees KR, Gemmill JD, Hillis WS, Reid JL (1991).
Transpulmonary pharmacokinetics of an ACE inhibitor (perindoprilat) in man.
Br.J.Clin.Pharmacol. 32: 193-199.

MacFadyen RJ, Lees, KR, Reid JL (1991b).
Differences in first dose response to angiotensin converting inhibition in congestive heart failure: a placebo controlled study. Br.Heart J. 66: 206-211.

MacFadyen RJ, Lees, KR, Reid JL (1993).
A double-blind, placebo controlled study of low dose intravenous perindoprilat or enalaprilat infusion in elderly patients with heart failure. Br.Heart J. 69: 293-297.

Mancini DM, Coyle E, Coggan A, et al (1989).
Contribution of intrinsic skeletal muscle changes to 31P NMR skeletal muscle abnormalities in patients with chronic heart failure. Circulation 80: 1338-1346.

Mancini DM, Henson D, La Manca J, Levine S (1992).
Respiratory muscle function and dyspnoea in patients with chronic congestive heart failure. Circulation 86: 909-918.

Marakas SA, Kyriakidis MK, Vourlioti AN, Petropoulakis PN, Toutouzakas PK (1993).
Acute effects of captopril administration on baroreflex sensitivity in patients with acute myocardial infarction. Eur. Heart J. 14: 1519-1523.

Marantz PR, Tobin JN, Wassertheil-Smoller S, et al. (1988).
The relationship between left ventricular systolic function and heart failure diagnosed by clinical criteria. Circulation 77: 607-612.

Marchetti J, Roseau S, Alhenc-Gelas F (1987).
Angiotensin I converting enzyme and kinin hydrolysing enzymes along the rabbit nephron.
Kidney Int. 31: 744-751.

Marre M, Chatellier G, Leblanc H, Guyene TT, Menard J, Passa P (1988).
Prevention of diabetic nephropathy with enalapril in normotensive diabetics with

microalbuminuria. Br.Med.J. 297: 1092-1095.

Maskin CS, Ocken S, Chadwick B, LeJemtel TH (1985).
Comparative systemic and renal effects of dopamine and angiotensin converting enzyme inhibition with enalaprilat in patients with heart failure.
Circulation 72: 846-852.

Massie B, Bourassa M, DiBianco R, et al (1985).
Long-term oral amrinone for congestive heart failure: lack of efficacy in a multicentre controlled trial. Circulation 71: 963-971.

Massie BM, Conway M, Yonge R, Frostick S, Sleight P, Ledingham J et al (1987).
31P nuclear magnetic resonance evidence of abnormal skeletal muscle metabolism in patients with congestive heart failure. Am.J.Cardiol. 60: 309-315.

Massie BM, Conway M, Yonge R, et al (1988).
Skeletal muscle metabolismin patients with in congestive heart failure: relation to clinical severity and blood flow. Circulation 76: 1009-1019.

Mathiesen ER, Hommel E, Giese J, Parving H-H (1991).
Efficacy of captopril in postponing nephropathy in normotensive insulin dependent diabetic patients with microalbuminuria. Br.Med.J. 303: 81-87.

McKee PA, Castelli WP, McNamara, Kannel WB (1971).
The natural history of congestive heart failure: The Framingham Study.
N.Eng.J.Med. 285: 1441-1446.

McLay JS, McMurray J, Bridges A, Struthers AD (1992).
Practical issues when initiating captopril therapy in chronic heart failure.
Eur.Heart J. 13: 1521-1527.

McMurray J, McDevitt DG (1990).
Treatment of heart failure in the elderly. Br.Med.Bull. 46: 202-229.

McMurray J, Lang CC, Maclean DD, McDevitt DG, Struthers AD (1989).
A survey of current use of angiotensin conveting enzyme inhibitors by Scottish physicians in the treatment of chronic cardiac failure. Scott.Med.J. 34: 425-427.

McMurray J, Dargie HJ (1992).
Trends in hospitalisation for chronic heart failure in the United Kingdom.
Eur.Heart J. 13(Suppl); 350-354.

McMurray J, Hart W, Rhodes G (1993).
An evaluation of the cost of heart failure to the National Health Service in the U.K.
Brit.J.Med.Econ. 6: 99-104.

Melbourne Diabetic Nephropathy Study Group (1991).
Comparison between perindopril and nifedipine in hypertensive and normotensive diabetic

patients with microalbuminuria. Br.Med.J. 302: 210-216.

Meiler SE, Ashton JJ, Moeschberger ML, Unverferth DV, Leier CV (1987).
An analysis of the determinants of exercise performance in congestive heart failure.
Am.Heart J. 113: 1207-1117.

Menard J, Bouhnik J, Clauser E, Richoux JP, Corvol P (1983).
Biochemistry and regulation of angiotensinogen. Clin. Exp. Hypertension. A5: 1005-1019.

Mendelsohn FAO (1984).
Localisation of angiotensin converting enzyme in art forebrain and other tissues by in vitro autoradiography. Clin.Exp.Pharm.Phys. 11: 431-436.

Mendelsohn FAO, Pupic V, Jackson B, Cubela R, Johnston C (1991).
Acute and chronic effects of perindopril on tissue angiotensin converting enzyme activity.
Am.J.Hypertens. 4: 220S-225S.

Merrill AJ, Morrison JC, Bannon ES (1946).
Concentration of renin in renal venous blood in patients with chronic heart failure.
Am. J. Med. 1: 468-472.

Michel JB, Lattion AL, Salzmann JL, Cerol M de L, Phillipe M, Camilleri JP, Corvol P (1988).
Hormonal and cardiac effects of converting enzyme inhibition in rat myocardial infarction.
Circ. Res. 62: 641-650.

Middlekauf HR, Stevenson WG, Warner-Stevenson L (1991).
Prognostic significance of atrial fibrillation in advanced heart failure.Circulation 84: 40-48.

Milani RV, Mehra MR, Reddy TK, Lavie CJ, Ventura HO (1996).
Ventilation/ carbon dioxide production ratio in early exercise predicts poor functional capacity in congestive heart failure. Heart 76: 393-396.

Misono KS, Halladay LA, Murakami K, Kuromizu K, Inagami T (1982).
Rapid and large scale purification and characterisation of renin from mouse submaxillary gland. Arch. Biochem Biophys. 217: 574-581.

Missouris CG, MacGregor GA (1996).
Survey of the use of angiotensin converting enzyme inihibtors in the treatment of congestive heart failure in hospital practice. (Abstract). Eur.Heart J. 17: (Suppl.): P406

Miyamori I, Yasuhara S, Takeda Y, Koshida H, Ikeda M, Nagai K, Okamoto H, Morise T, Takeda R, Aburano T (1986).
Effects of converting enzyme inhibition on split renal function in renovascular hypertension. Hypertension 8: 415-421.

Miyamori I, Yasuhara S, Matsubara T, Takasaki H, Takeda R (1988).
Comparative efefcts of captopril and nifedipine on split renal function in renovascular

hypertension. Am.J.Hypertens. 1: 359-363.

Modina N, Packer M, Lee WH, Yushak M (1986).
Comparison of captopril and enalapril in patients with severe chronic heart failure.
N.Eng.J.Med. 315: 847-853.

Mogensen CE (1984).
Long term antihypertensive treatment inhibiting progresion of diabetic nephropathy.
Br.Med.J. 285: 685-688.

Mogensen CE (1984).
Microalbuminuria predicts clinical proteinuria and early mortality in maturity onset
diabetes. N.Engl.J.Med. 310: 356-360.

Mohanty N, Wasserman A, Walker P, Katz RJ (1995).
Prevention of nitroglycerin tolerance with diuretics. Am. Heart J. 130: 522-57.

Mombouli JV, Nephtali M, Vanhoutte PM (1991).
Effects of the non-sulfhydryl angiotensin I converting enzyme inhibitor cilazaprilat on
endothelial dependent responses in isolated canine arteries.
Hypertension 18 (Suppl. II): II22-29.

Moore DP, Weston A, Hughes JM, Oakley CM, Cleland JGF (1993).
Bronchial hyperresponsiveness in heart failure. N. Engl. J. Med. 328: 1424-1425.

Moravec C, Schuluchter MD, Paranandi L, Czerska B, Stewart RW, Rosenkranz E, Bond
M et al (1990).
Inotropic effects of angiotensin II on human cardiac muscle in vitro.
Circulation 82: 1973-1984.

Morisco C, Cuocolo A, Romano M, Nappi A, Iaccarino G, Volpe M, Salvatore M,
Trimarco B (1996).
Influence of digitalis on left ventricular functional response to exercise in congestive heart
failure. Am.J.Cardiol. 77: 480-485.

Morton JJ, Tree M, Casals-Stenzel J (1980).
The effect of captopril on blood pressure and angiotensins I, II and III in sodium-depleted
dogs: problems associated with the measurement of angiotensin II after inhibition of
converting enzyme. Clin.Sci. 58: 445-450.

Motwani JG, Fenwick MK, Morton JJ, Struthers AD (1992).
Furosemide-induced natriuresis is augmented by ultra-low dose captopril but not by
standard doses of captopril in chronic heart failure. Circulation 86: 439-445.

Motwani JG, Fenwick MK, Morton JJ, Struthers AD (1994).
Determinants of the initial effects of captopril on blood pressure, glomerular filtration rate
and natriuresis in mild-to-moderate congestive heart failure secondary to coronary artery
disease. Am.J.Cardiol. 73: 1191-1196.

Mujais SK, Fouad FM, Textor SC, et al. (1984).
Transient renal dysfunction during initial inhibition of converting enzyme in congestive heart failure. Br.Heart J. 52: 63-71.

Mulligan IP, Fraser AG, Lewis MJ, Henderson AH (1989).
Effects of enalapril on myocardial noradrenaline outflow during exercise in patients with chronic heart failure. Br.Heart J. 61: 23-28.

Mullins JJ, Peters J, Ganten D (1990).
Fulminant hypertension in transgenic rats harbouring the mouse Ren-2 gene.
Nature 344: 541-544.

Munger MA, Benotti JR, Green ja et al (1990).
Assessment of haemodynamic tolerance from a 24 hour intravenous infusion of fenoldapam mesylate in congestive heart failure. Am.J.Cardiol. 65: 206-210.

Munzel T, Drexler H, Holtz J, Kurtz S, Just H (1991).
Mechanisms involved in the response to prolonged infusion of atrial natriuretic factor in patients with chronic heart failure. Circulation 83: 191-201.

Myers J, Froelicher V (1991).
Haemodynamic determinants of exercise capacity in chronic heart failure.
Ann.Int.Med. 115: 377-386.

Naftilan AJ, Zou WM, Ingelfiger JR, Ryan TJ, Pratt Re, Dzau VJ (1991).
Localisation and differential regulation of angiotensinogen mRNA expression in the vessel wall. J.Clin. Invest. 97: 1300-1311.

Naftilan AJ (1992).
The role of angiotensin II in vascular smooth muscle cell growth. J.Cardiovasc. Pharmacol. 20: S37-S40.

Nakajima Y, Yamada T, Setoguchi M. (1992).
Prolonged inhibition of local angiotensin converting enzyme after single or repeated treatment with quinapril in spontaneously hypertensive rats.
J.Cardiovasc.Pharmacol. 19: 102-107.

Narang R, Cleland JGF, Ball S, et al. (1996).
Mode of death in chronic heart failure: a request for more accurate classification.
Eur.Heart J. 17: 1390-1403.

Naruse M, Takii Y, Inagami T (19810.
Renin exists in high concentration in the adrenal gland of rat. Biomed. Res. 2: 583-586.

Ng KKF, Vane JR (1968).
Fate of angiotensin I in the circulation. Nature 218: 144-150

Nicholls MG, Espiner EA, Hughes H, Rogers T (1976).
Effect of potassium-sparing diuretics on the renon-angiotensin-aldosterone system and potassium retention in heart failure. Br.Heart J. 38: 1025-1030.

Nieminen Ms, Kupari M (1990).
The haemodynamic effects of ACE inhibitors in the treatment of congestive heart failure. J.Cardiovasc.Pharmacol.15 (Suppl 2: S36-S40.).

Nonogi H, Hess OM, Bortone AS, Ritter M, Carroll JD, Krayenbuehl HP (1989).
Left ventricular pressure-length relation during exercise induced ischaemia. J.Am.Coll.Cardiol. 13: 1062-1070.

Nussberger J, Brunner DB, Waeber B, Brunner HR (1984).
Measurement of low angiotensin concentrations after ethanol and Dowex extraction procedures. J.Lab.Clin.Med 103: 304-312.

Nussberger J, Brunner DB, Waeber B, Brunner HR (1985).
True versus immunoreactive angiotensin II in human plasma. Hypertension 7(Suppl.I): I1-I7.

Nussberger J, Brunner DB, Waeber B, Brunner HR (1986).
Specific measurement of angiotensin metabolites and in vitro generated angiotensin II in plasma. Hypertension 8: 476-482.

Nussberger J, Brunner DB, Waeber B, Brunner HR (1988).
In vitro renin inhibition to prevent generation of angiotensins during determination of angiotensin I and II. Life Sci. 42: 1683-1688.

Nussberger J, Keller I, Waeber B, Brunner HR (1988).
Angiotensin II measurement with high affinity monoclonal antibodies. J.Hypertension 6 (Suppl 4): S424-425.

Nussberger J, Juillerat L, Perret F, Waeber B, Bellet M, Brunner J, Menard J (1989).
Need for plasma angiotensin measurements to investigate converting enzyme inhibition in humans. Am. Heart J. 117: 717-722.

Nussberger J, Fleck E, Bahrmann H, Delius W, Schultheiss HP, Brunner HR (1994).
Dose-related effects of ACE inhibition in man: quinapril in patients with moderate congestive heart failure. Eur. Heart J. 15 (Suppl D): 113-122.

O'Donnell MP, Kasiske BL, Katz SA, Schmitz PG, Keane WF (1989).
Enalapril reduces glomerular injury in obese Zucker rats. Kidney Int. 35: 434.

Okamura T, Ikunishi H, Ayajiki K, Tona N (1990).
Conversion of angiotensin I to angiotensin II in dog isolated renal artery: role of two different angiotensin II generating enzymes. J.Cardiovasc.Pharmacol. 15: 353-359.

Oliver JA, Sciacca RR (1984).

Local generation of angiotensin II as a mechanism of peripheral vascular tone in the rat. J.Clin. Invest. 74: 1247-1251.

Olsen SL, Gilbert EM, Renlund DG, Taylor DO, Yanowitz FD , Bristow MR. (1995). Carvedilol improves symptoms and left ventricular function and symptoms in chronic heart failure: a double-blind randomised study. J.Am.Coll.Cardiol. 25: 1225-1231.

Olson SC, Harvath AM, Sedman AJ, Colburn WA, Welling PG (1989). The clinical pharmacokinetics of quinapril. Angiology; 40: 351-359.

O'Neill CJA, Bowes SG, Sullens CM, et al. (1988). Evaluation of the safety of enalapril in the tretament of heart failure in the elderly. Eur.J.Clin.Pharmacol. 35: 143-150.

OPC-8212 Multicenter Research Group (1990). A placebo-controlled, randomised, double-blind study of vesnarinone in patients with mild chronic heart failure. Cardiovasc.Drugs Ther. 4: 419-426.

Osterziel KJ, Dietz R, Schmid W, Mikulaschek K, Manthey J, Kubler W (1990). ACE inhibition improves vagal reactivity in patients with heart failure. Am.Heart J. 120: 1120-1129.

Oliver RM, Peacock AJ, Fleming JS, Waller DG (1989). Renal and pulmonary effects of angiotensin converting enzyme inhibition in chronic hypoxic lung disease. Thorax 44: 513-515.

Owens GK (1987). Influence of blood pressure on the development of aortic medial smooth muscle hypertrophy in spontaneously hypertensive rats. Hypertension 9: 178-187.

Packer M, Medina N, Yushak M, Meller J (1983). Hemodynamic patterns of response during long-term captopril therapy for severe chronic heart failure. Circulation 68: 803-812.

Packer M, Medina N, Yushak M, Lee WH (1984). Correction of dilutional hyponatraemia in severe chronic heart failure by converting-enzyme inhibition. Ann. Int. Med. 100: 782-789.

Packer M (1985). Sudden unexpected death in patients with congestive heart failure: a second frontier. Circulation 72: 681-685.

Packer M, Medina N, Yushak M, Lee WH (1985). Usefulness of plasma renin activity in predicting haemodynamic and clinical responses and survival during long term converting enzyme inhibition in severe chronic heart failure: experiences in 100 consecutive patients. Br. Heart J. 54: 298-304.

Packer M, Lee WH (1986).

Provocation of hyper- and hypokalaemic sudden death during treatment with and withdrawal of converting enzyme inhibition in patients with severe chronic heart failure. Am.J.Cardiol. 57: 347-348.

Packer M, Kessler P, Gottlieb SS (1986).
Adverse effects of converting enzyme inhibition in patients with severe congestive heart failure: pathophysiology and management. Postgrad. Med.J. 62 (Suppl.1): 179-182.

Packer M, Lee WH, Kessler PD (1986a).
Preservation of glomerular filtration rate in human heart failure by activation of the renin angiotensin system. Circulation 74: 766-774.

Packer M, Lee WH, Yushak M, Medina N (1986b).
Comparison of captopril and enalapril in patients with severe chronic heart failure. N.Engl.J.Med. 315: 847-853.

Packer M, Lee WH, Kessler PD, Gottlieb SS, Bernstain JL, Kukin ML (1987a).
Role of neurohormonal mechanisms in determining survival in patients with severe chronic heart failure. Circulation 75 (Suppl. IV): 80-92.

Packer M, Lee WH, Kessler PD, Gottlieb SS, Medina N, Yushak M (1987b).
Prevention and reversal of nitrate tolerance in patients with congestive heart failure. New Engl.J.Med. 317: 799-804.

Packer M, Leier CV. (1987).
Survival in congestive heart failure during treatment with drugs with positive inotropic actions. Circulation 75 (Suppl. IV): IV55-IV63.

Packer M (1988).
Clinical trials in congestive heart failure: why do studies report conflicting results? Ann.Int.Med. 109: 3-5.

Packer M (1989).
Identification of risk factors predisposing to the development of functional renal insufficiency during treatment with converting enzyme inhibitors in chronic heart failure. Cardiology 76 (Suppl. 2): 50-55.

Packer M (1990).
The placebo effect in heart failure. Am.Heart J. 120: 1579-1582.

Packer M (1990).
Calcium channel blockers in chronic heart failure. Circulation 82: 2254-2257.

Packer M, Carver Jr, Rodeheffer RJ, et al (1991).
Effect of oral milrinone on mortality in severe chronic heart failure. N.Engl.J.Med. 325: 1468-1475.

Packer M, Gheorghiade M, Young JB, et al (1993).

Withdrawal of digoxin from patients with chronic heart failure treated with angiotensin converting enzyme inhibitors. N.Eng.J.Med. 329: 1-7.

Packer M, O'Connor CM, Ghali JK, et al (1996).
Effects of amlodipine on morbidity and mortality in severe chronic heart failure.
N.Eng.J.Med. 335: 1107-1114.

Parameshwar J, Schackell Mm, Richardson A, Poole-wilson Pa, Sutton GC (1992).
Prevalence of heart failure in three general practices in north west London.
Br.J.Gen.Pract. 42: 287-289.

Paquet JL, Bandonim-Legros M, Brunelle G, Meyer P (1990).
Angiotensin II-induced proliferation of aortic myocytes in spontaneously hypertensive rats.
J.Hypertension 8: 565-572.

Parving H-H, Anderson AR, Smidt UM, Hommel E, Mathiesen ER, Svendsen PA (1987).
Effects of antihypertensive treatment on kidney function in diabetic nephropathy.
Br.Med.J. 294: 1443-1447.

Parving H-H, Hommel E, Smidt UM (1988).
Protection of kidney function and decrease in albuminuria by captopril in insulin dependent diabetics with nephropathy. Br.Med.J. 297: 1068-1091.

Pasa P, LeBlanc H, Marre M (1987).
Effects of enalapril in insulin dependent diabetic subjects with mild to moderate uncomplicated hypertension. Diabetes Care 10: 200-204.

Pedersen MM, Schmitz A, Pedersen EB, Danielson H, Christiansen JS (1988).
Acute and long term renal effect of angiotensin converting enzyme inhibition in normotensive, normoalbuminuric insulin dependent diabetic patients.
Diab.Med. 5: 562-569.

Pelliccia F, Borghi A, Ruggeri A, Cianfrocca C, Morgagni GLl, Bugiardini R (1993).
Changes in pulmonary haemodynamics predict benefits in exercise capacity after ACE inhibition in patients with mild to moderate congestive heart failure.
Clin. Cardiol. 16: 607-612.

Pfeffer MA, Pfeffer JM, Fishbein MC, Fletcher PJ, Spadaro J, Kloner RA, Braunwald E (1979).
Myocardial infarct size and ventricular function in rats. Circ.Res. 44: 503-512.

Pfeffer JM, Pfeffer MA, Braunwald E (1985).
Influence of chronic captopril therapy on the infarcted left ventricle of the rat.
Circ.Res. 57: 84-95.

Pfeffer MA, Lamas GA, Vaughan DE, Parisi DF, Braunwald E (1988)
Effect of captopril on progressive dilatation after anterior myocardial infarction.
New Engl. J. Med. 319: 80-86.

Pfeffer MA, Braunwald E, Moye LA, et al. on behalf of the SAVE investigators (1992).
Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction. New Engl.J.Med. 327: 669-677.

Philbin EF, Andreou C, Rocco TA, Lynch LJ, Baker SL (1996).
Patterns of angiotensin converting enzyme inhibitor use in congestive heart failure in two community hospitals. Am.J.Cardiol. 77: 832-838.

Piquilloud Y, Reinharz A, Roth M (1970).
Studies on the angiotensin converting enzyme with different substrates.
Biochem. Biophys. Acta 206: 136-142.

Poole-Wilson PA (1988).
The origin of symptoms in patients with heart failure. Eur.Heart J. 9(Suppl.H): 49-53.

Postma CT, Hoefnagels WH, Barentsz JO, de Boo T, Thien T (1989).
Occlusion of unilateral stenosed renal arteries. Relation to medical treatment.
J.Human. Hypertens. 2: 185-190.

Pouleur H, Hanet C, Rousseau MF, Van Eyll C (1990).
Relation of diastolic function and exercise capacity in ischaemic left ventricular dysfunction. Role of beta agonists and beta antagonists. Circulation 82 (Suppl.): 189-196.

Puri S, Baker BL, Oakley CM, Hughes CMB, Cleland JGF (19940.
Increased alveolar-capillary membrane resistance to gas transfer in patients with chronic heart failure. Br. Heart J. 72: 140-144.

Rajagopalan B, Conway MA, Massie B, Radda GK (1988).
Alterations of skeletal muscle metabolism in humans studied by phosphorous 32 magnetic resonance spectroscopy in congestive heart failure.
Am.J.Cardiol. 62: 53E-57E.

Rajfer SI (1993).
Perspective of the pharmaceutical industry on the development of new drugs for heart failure. J.Am.Coll.Cardiol. 22(suppl A): 198A-200A.

Rapp JP, Wang SM, Dene H (1989).
A genetic polymorphism in the renin gene of Dahl rat cosegregates with blood pressure.
Science 243: 542-544.

Re R, Fallon JT, Dzau V, Quay SC, Haber E (1982).
Renin synthesis by canine aortic smooth muscle cells in culture. Life Sci. 30: 99-106.

Reams GP, Souther M, Parisi M, van Stone J, Bauer JH (1989).
Angiotensin peptides in human subjects. J.lab.clin.Med. 113: 749-752.

Regitz-Zagrosek V, Leuchs B, Krulls-munch J. (1995).

Angiotensin converting enzyme inhibitors and β-blockers in long-term treatment of dilated cardiomyopathy. Am.Heart J. 129: 754-761.

Remes J, Reunanen A, Aromaa A, Pyorala K (1992).
Incidence of heart failure in eastern Finland: a population based surveillance study.
Eur. Heart J. 13: 588-593.

Riegger GAJ (1990).
The effects of ACE inhibitors on exercise capacity in the treatment of congestive heart failure. J.Cardiovasc.Pharmacol. 15 (Suppl. 2): S41-S46.

Reineck HJ, Kreisberg JL (1983).
Renal vascular response to angiotensin II in rats with streptozotocin-induced diabetes mellitus. Kidney Int. 24: 247 (Abstract).

Reitsma JB, Mosterd A, de Craen AJM, Koster RW, van Capelle FJL, Grobbee DE, Tijssen JGP (1996).
Increase in hospital admission rates for heart failure in the Netherlands, 1980-1993.
Heart 76: 388-392.

Remes J, Reunanen A, Arounaa A, Pyorala K (1992).
Incidence of heart failure in eastern Finland: a population based study.
Eur.Heart J. 13: 588-593.

Reydel-Bax P, Redalieu E, Raklit A.
Direct determination of angiotensin inhibitors in plasma by radioenzymatic assay.
Clin.Chem. 1987 33/34: 549-553.

Ribstein J, Mourad G, Mimran A (1988).
Contrasting acute effects of captopril and nifedipine on renal function in renovascular hypertension. Hypertension 1: 239-244.

Richards AM, Cleland JGF, Tonolo G, McIntyre GD, Leckie BJ, Dargie HJ, Ball SG, Robertson JIS (1986).
Plasma α natriuretic peptide in cardiac impairment. Br.Med.J. 293: 409-412.

Richards AM, Nicholls MG, Lewis L, Lainchbury JG (1996).
Adrenomedullin. Clinical Science 91: 3-16.

Riegger AJG, Elsner D, Kromer EP, et al. (1988).
Atrial natriuretic peptide in congestive heart failure in the dog: plasma levels, cyclic guanosine monophosphate, ultrastructure of atrial myoendocrine cells, and haemodynamic, hormonal and renal effects. Circulation 77: 398-406.

Robertson JIS, Richards AM (1987).
Converting enzyme inhibitors and renal function in cardiac failure.
Kidney Int. 31 (Suppl. 20): S216-219.

Rockman HA, Juneau C, Chatterjee K, Rouleau J-L (1989).
Long-term predictors of sudden and low output death in chronic congestive heart failure
secondary to coronary artery disease. Am.J.Cardiol. 64: 1344-1348.

Rogerson FM, Chai S-Y, Schlawe I, Murray WK, Marley PD, Mendelsohn FAO (1992).
Presence of angiotensin converting enzyme in the adventitia of large blood vessels.
J. Hypertension 10: 615-620.

Rogg H, de gasparo M, Graedel E, Stulz P, Burkart F, Eberhard M, Erne P (1996).
Angiotensin-II receptor subtypes in human atria and evidence for alterations in patients
with cardiac dysfunction. Eur.Heart J. 17: 1112-1120.

Rosenthal J, von Lutterotti N, Thurnreiter M, et al (1987).
Suppression of renin angiotensin system in the heart of spontaneously hypertensive rats.
J.Hypertension 5 (Suppl 2): S23-S31.

Rosenthal J, Thurnretier M, Plaschdke M, Geyer M, Reiter W, Dahlheim H (1990).
renin-like enzymes in human vasculature. Hypertension 15: 848-853.

Ryan JW, Chung A, Ammons C, Carlton ML (1977).
A simple radioassay for angiotensin converting enzyme. Biochem. J. 167: 501-504.

Ryan JW, Chung AYK, Berryer P, Murray MA, Ryan JPA (1986).
Slow tight binding inhibitors of angiotensin converting enzyme.
Ad.Exp.Med.Biol. 198: 419-425.

Said SI (1982).
Metabolic functions of the pulmonary circulation. Circ. Res. 50: 325-333.

Sakaguchi K, Chai SY, Jackson B, Johnston CI Mendelsohn FA (1988).
Inhibition of tissue angiotensin converting enzyme. Quantitation by autoradiography.
Hypertension 11: 230-238.

Salmon P, Brown MA (1990).
Renal artery stenosis and peripheral vascular disease: implications for ACE inhibitor
therapy. Lancet 336: 321.

Samani NJ, Swales JD, Brammar WJ (1990).
A widespread abnormality of renin gene expression in the spontaneously hypertensive rat:
modulation in some tissues with the development of hypertension.
Clin.Sci. 77: 629-636.

Sano S, Tarazi RC (1987).
Differential structural responses of small resistance vessels to antihypertensive therapy.
Hypertension 75: 618-626.

Sapsford RJ, Hall AS, Robinson MB (1996).
Evaluation of angiotensin converting enzyme inhibitor dosage and prescription rates in

post myocardial infarction patients. Eur.Heart J. 17: (Suppl.): P411

Sato H, Hori M, Ozaki H, et al (1993).
Exercise induced upward shift of diastolic left ventricular pressure-volume relationship in patients with dilated cardiomyopathy-Effect of β-adrenoceptor blockade.
Circulation 88: 2215-2223.

Schalekamp MADH, Admiraal PJJ, Derkx (1989).
Estimation of regional metabolism and production of angiotensins in hypertensive subjects.
Br.J.Clin.Pharmac. 28: 105S-113S.

Schaufelberger M, Eriksson BO, Held P, Swedberg K (1996).
Skeletal muscle metabolism during exercise in patients with chronic heart failure.
Heart 76: 29-34.

Schelling P, Fischer H, Ganten D (1991).
Angiotensin and cell growth: a link to cardiovascular hypertrophy ?
J.Hypertension 9: 3-15.

Schmitz A, Christensen T, Moller A, Mogensen CE (1990).
Kidney function and cardiovascular risk factors in non-insulin dependent diabetics (NIDDM) with microalbuminuria. J.Intern.Med. 228: 347-352.

Schocken D, Arrieta MI, Leaverton PE (1992).
Prevalence and mortality rate of congestive heart failure in the United States.
J.Amer.Coll.Cardiol. 20: 301-306.

Scott-Burden T, Resink TJ, Hahn AWA, Buhler FR (1991).
Angiotensin induced growth-related metabolism is activated in cultured smooth muscle cells from spontaneously hypertensive rats and Wistar-Kyoto rats.
Am.J.Hypertension 4: 183-188.

Sedman AJ, Posvar E (1989).
Clinical pharmacology of quinapril in healthy volunteers and in patients with hypertension and congestive heart failure. Angiology 40: 360-366.

Semple PFS, Boyd AS, Dawes PM, Morton JJ (1976).
Angiotensin II and its heptapeptide (2-8), hexapeptide (3-8) and pentapeptide (4-8) metabolites in arterial and venous blood of man. Circ. Res. 39: 671-678.

Semple PFS, Thoren P, Lever AF (1988).
Vasovagal reactions to cardiovascular drugs: the first dose effect.
J.Hypertension 6: 601-606.

Sharpe N, Murphy J, Smith H, Hannan S (1988).
Treatment of patients with symptomless left ventricular dysfunction after myocardial infarction. Lancet 1: 255-259.

Sharpe N, Smith H, Murphy J, Greaves S, Hart H, Gamble G (1991).
Early prevention of left ventricular dysfunction after myocardial infarction with angiotensin converting enzyme inhibition. Lancet 337: 872-876.

Shioto N, Miyazaki M, Okunishi H (1992).
Increase of angiotensin converting enzyme gene expression in the hypertensive aorta. Hypertension 20: 168-174.

Siekely MG, Arant BS Jr, Seney FD Jr (1990).
Endogenous angiotensin concentrations in specific intrarenal fluid compartments of the rat. J.Clin.Invest. 86: 1352-1357.

Simon AC, Leverson JA, Bouthier DJ, Maarek BC, Safar ME (1985).
Effects of acute and chronic angiotensin converting enzyme inhibition on large arteries in human hypertension. J.Cardiovasc.Pharmacol. 7 (Suppl.): 45-51.

Slazchcic J, Massie BM, Kramer BL, Topic N, Tuban J (1985).
Correlates and prognostic implications of exercise capacity in chronic congestive heart failure. Am. J. Cardiol. 55: 1037-1042.

Sogaard P, Gotzsche CO, Ravkilde J, Norgaard A, Thygesen K (1994).
Ventricular arrythmias in the acute and chronic phases after acute myocardial infarction: effect of intervention with captopril. Circulation 90: 101-107.

Squire IB , Reid JL (1993).
Interactions Between the Renin-Angiotensin System and the Autonomic Nervous System. IN: The Renin Angiotensin System: J.I.S. Robertson, M.G.Nicholls, Editors. Gower Medical Publishing, London.

Staessen J, Wilms G, Baert A, Fagard R, Lijnen P, Suy R, Amery A (1988).
Blood pressure during long-term converting-enzyme inhibition predicts the curability of renovascular hypertension by angioplasty. Am.J.Hypertens. 1: 208-214.

Stornello M, Valvo EV, ScapellatoL (1989).
Hemodynamic, renal and and humoral effects of the calcium entry blocker nicardipine and converting enzyme inhibitor captopril in hypertensive type II diabetic patients with nephropathy. J.Cardiovasc.Pharmacol. 14: 851-855.

Stornello M, Valvo EV, ScapellatoL (1991).
Comparative effects of enalapril, atenolol and chlorthalidone on blood pressure and kidney function of diabetic patients affected by arterial hypertension and persistent proteinuria. Nephron 58: 52-57.

Stornetta RL, Hawelu-Johnson CL, Guyenet PG, Lynch KR (1988).
Astrocytes synthesise angiotensinogen in brain. Science 242: 1444-1446.

Struthers AD (1993).
Plasma concentrations of brain natriuretic peptide : will this new test reduce the need for

cardiac investigations ? Br.Heart J. 70: 397-398.

Struthers AD, Whitesmith R, Reid JL (1983).
Prior thiazide diuretic treatment increases adrenaline induced hypokalaemia.
Lancet 1: 1358-1361.

Sullivan MJ, Higginbotham MB, Cobb FR (1988).
Increased exercise ventilation in patients with chronic heart failure: intact ventilatory
control despite haemodynamic and pulmonary abnormalities. Circulation 77: 552-559.

Sullivan M, Atwood JE, Myers J, Feuer J, Hall P, Kellerman B, Forbes S, Froelicher V
(1989).
Increased exercise capacity after digoxin administration in patients with heart failure.
J.Am.Coll.Cardiol. 13: 1138-1143.

Sullivan MJ, Knight JD, Higginbotham MB, Cobb FR (1989).
Relation between central and peripheral haemodynamics during exercise in patients with
chronic heart failure. Muscle blood flow is reduced with maintenance of arterial perfusion
pressure. Circulation 80: 769-781.

Sullivan MJj, Green HJ, Cobb FR (1990).
Skeletal muscle biochemistry and histology in ambulatory patients with long-term
heart failure. Circulation 81: 518-527.

Sutton GC (1990).
Epidemiologic aspects of heart failure. Am.Heart J. 120: 1538-1540.

Swedberg K, Held P, Kjekshus J, et al. (1992).
Effects of early administration of enalapril on mortality in patients with acute myocardial
infarction. Results of the Cooperative North Scandinavian Enalapril Survival Study II
(CONSENSUS II). N.Engl.J.Med. 327: 678-684.

Swartz SL, Williams GH, Hollenberg NK, Moore TJ, Dluhy RG (1979).
Converting enzyme in essential hypertension: the hypotensive response does not reflect
only reduced angiotensin II formation. Hypertension 1: 106-111.

Swedberg K, Eneroth P, Kjekshus J, Wilhelmsen L (1990).
Hormones regulating cardiovascular function in patients with severe congestive heart
failure and their relation to mortality. Circulation 82: 1730-1736.

Tang SS, Stevenson L, Dzau VJ (1990).
Endothelial renin-angiotensin pathway. Circ.Res. 66: 103-108.

Tani M, Fujika A, Asanoi H, et al.(1992)
Effects of chronotropic responsive cardiac pacing on ventilatory response to exercise in
patients with complete AV block. P.A.C.E. 15: 1482-1491.

Textor SC, Novick RC, Tarazi AC, Klimas V, Vidt DG, Pohl M (1985).

Critical perfusion pressure for renal function in patients with bilateral atherosclerotic renal vascular disease. Ann.Int.Med.102: 308-314.

The AIRE Study Investigators.
Effect of ramipril on mortality and morbidity of survivors of acute myocardial infarction with clinical evidence of heart failure. Lancet 1993 342: 821-828.

The CONSENSUS Trial Study Group.
Effect of enalapril on mortality in severe congective cardiac failure: results of the Cooperative North Scandinavian Trial Group. N.Eng.J.Med. 1987 316: 1429-1435.

The Pimobendan in Congestive Heart Failure (PICO) Investigators (1996).
Effects of pimobendan on exercise capacity in patients with heart failure: main results from the Pimobendan in Congestive Heart Failure (PICO) trial. Heart 76: 223-231.

The SAVE Investigators (1992).
Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction: results of the Survival and Ventricular Enlargement Trial. N.Eng.J.Med. 327: 669-677.

The SOLVD Investigators (1991).
Effect of enalapril on survival in patients with reduced left ventricular ejection fraction and congestive heart failure. N.Eng.J.Med. 295: 293-302.

The SOLVD Investigators (1992).
Effect of enalapril on mortality and the development of heart failure in asymptomatic patients with reduced left ventricular ejection fraction. N.Eng.J.Med. 327: 685-691.

The Xamoterol In Severe Heart Failure Study Group (1990).
Xamoterol in severe heart failure. Lancet 336: 1-6.

Tigerstedt R, Bergman PG (1898).
Niere und Kreislauf. Skandinavisches fur Physiologie 8: 223-271.

Thibault G, Genest J (1981).
Tonin, an esteroprotease from rat submaxillary glands. Biochem.Biophys.Acta 660: 23-29.

Todd PA, Heel RC (1986).
Enalapril: a review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in hypertension and congestive heart failure. Drugs 31:198-248.

Tohmeh JF, Cryer PE. (1980).
Biphasic adrenergic modulation of β-adrenergic receptors in man: agonist early increment and late decrement of β-adrenergic receptor number. J.Clin.Invest. 65: 836-840.

Tonnesen MG, Klempner MS, Austen KF, Wintroub BU (1982).
Identification of a human neutrophil angiotensin II-generating protease as cathepsin G. J.Clin.Invest. 69: 25-30.

Tsuda T, Kawahara Y, Shii K, Koide M, Ishida Y, Yokoyama M (1991).
Vasoconstrictor induced protein-tyrosine phosphorylation in cultured vascular smooth muscle cells. FEBS Lett. 285: 44-48.

Tuchelt H, Eschenhagen G, Bahr V, Schwietzer G, Thiede HM, Oelkers W (1990).
Role of atrial natriuretic factor in changes in the responsiveness of aldosterone to angiotensin II secondary to sodium loading and depletion in man. Clin.Sci. 79: 57-65.

Ubeda M, Hernandez I, Fenoy FJ, Quesada T (1988).
Adrenal and vascular renion-like activity in chronic two-kidney, one clip hypertensive rats. Clin.Physiol. 6: 275-280.

Unger T, Ganten D, Lang RE, Scholkens BA (1984).
Is tissue converting enzyme inhibition a determinant of the antihypertensive efficacy of converting enzyme inhibitors ? Studies with the two different compounds Hoe 498 and MK421 in spontaneously hypertensive rats. J.Cardiovasc. Pharmacol. 6: 672-680.

Unger T, Ganten D, Lang RE, Scholkens BA (1985).
Persistent tissue converting enzyme inhibition following treatment with Hoe 498 and MK 421 in spontaneously hypertensive rats. J.Cardiovasc.Pharmacol. 7: 36-41.

Unger T, Ganten D, Lang RE (1986).
Tissue converting enzyme and cardiovascular actions of converting enzyme inhibitors. J.Cardiovasc.Pharmacol. 8 (Suppl 10): S75-S81.

Urata H, Healy B, Stewart RW, Bumpus FM, Husaina L(1989).
Angiotensin II receptors in normal and failing human hearts. J.Clin.Endocrinol.Metab. 69: 54-66.

Urata H, Kinoshita A, Misonon KS, Bumpus FM, Husain A (1990).
Identification of a highly specific chymase as the major angiotensin II forming enzyme in the human heart. J.Biol.Chem.
265: 22 348-22 357.

Uren NG, Davies SW, Agnew JE, et al (1993a).
Reduction in mismatch of global ventilation and perfusion on exercise is related to exercise capacity in chronic heart failure. Br. Heart J. 70: 241-246.

Uren NGg, Davies SW, Jordan SL, Lipkin DP (1993b).
Inhaled bronchodilators increase maximum oxygen consumption in chronic left ventricular failure. Eur. heart J. 14: 744-750.

Uretsky BF, Generalovich T, Verbalis JG, Valdes AM, Reddy PS (1986).
Comparative haemodynamic and hormonal response of enoximone and dobutamine in severe congestive heart failure. Am.J.Cardiol. 58: 110-116.

Uretsky BF, Jessup M, Konstam MA, et al (1990).

Multicentre trial of oral enoximone in patients with moderate to moderately severe congestive heart failure: lack of benefit compared to placebo.
Circulation 82: 774-780.

Uretsky BF, Young JB, Shahidi FE, Yellen LG, Harrison MC, Jolly MK (1993).
Randomised study assessing the effect of digoxin withdrawal in patients with mild to moderate chronic congestive heart failure: results of the PROVED study.
J.Am. Coll.Cardiol. 22: 955-962.

Waagstein F, Bristow Mr, Swedberg K, Camerinin F, Fowler MB, Silver MA, Gilbert EM, Johnson MR, Goss FG, Hjalmarson A. (1993).
Beneficial effects of metoprolol in idiopathic dilated cardiomyopathy.
Lancet 342: 1441-1446.

Wada O, Asanoi H, Miyagi K, et al. (1993).
Importance of abnormal lung perfusion in excessive exercise ventilation in chronic heart failure. Am. heart J. 125: 790-798.

Wadworth AN, Brogden RN (1991).
Quinapril: A review of its pharmacological properties, and therapeutic efficacy in cardiovascular disorders. Drugs 41(3): 378-399.

Waeber B, Brunner HR, Brunner DB, Curtet AL, Turini GA, Gavras H (1980).
Discrepancy between antihypertensive effect and angiotensin converting enzyme inhibition by captopril. Hypertension 2: 236-242.

Walsh JT, Andrews R, Evans A, Cowley AJ (1995).
Failure of "effective" treatment for heart failure to improve normal customary activity.
Br.Heart J. 73: 373-376.

Wasserman K, Casaburi R (1988).
Dyspnea: Physiological and pathophysiological mechanisms. Ann.Rev.Med. 39:503-515.

Weber KT, Janicki JS (1985).
Cardiopulmonary exercise testing for evaluation of chronic heart failure.
Am.J.Cardiol. 55: 22A-31A.

Weber KT, Janicki JS (1985).
Lactate production during maximal and submaximal exercise in patients with chronic heart failure. J.Am.Coll.Cardiol. 6: 717-724.

Webster MWI, Fitzpatrick MG, Nicholls MG, Ikram H, Wells JE (1985).
Effect of enalapril on ventricular arrhythmias in congestive heart failure.
Am.J.Cardiol. 56: 566-569.

Wei L, Alhenc-Gelas F, Corvol P, Clause E (1991).
The two homologous domains of human angiotensin converting enzyme are both catalytically active. J.Biol.Chem. 266: 9002-9008.

Weishaar RE, Panek RL, Major TC, Simmerman J, Rapundalo ST, Taylor DG (1991).
Evidence for a functional tisue renin-angiotensin system in the rat mesenteric vasculature and its involvement in regulating blood pressure.
J.Pharmacol.Exp.Ther. 256: 568-574.

Wenting GJ, De Bruyn JHB, Man In't Veld, AJ, et al (1982).
Haemodynamic effects of captopril in essential hypertension, renovascular hypertension and cardiac failure: correlations with short- and long-term effects on plasma renin.
Am.J.Cardiol. 49: 1453-1459.

West JNW, Smith SA, Stallard TJ, Littler WA (1989).
Effects of perindopril on ambulatory intra-arterial blood pressure, cardiovascular reflexes and forearm blood flow in essential hypertension. J.Hypertension 7: 97-104.

Wheeldon NM, MacDonald TM, Flucker CJ, McKendrick AD, McDevitt DG, Struthers AD (1993).
An echocardiographic study of chronic heart failure in the community. Q.J.Med 86: 17-23.

Wilson JR, Ferraro LN (1982).
Effect of isosorbide dinitrate on submaximal exercise capacity of patients with chronic left ventricular failure. Chest 82: 701-704.

Wilson JR, Ferraro LN (1985).
Effects of the renin-angiotensin system on limb circulation and metabolism during exercise in patients with heart failure. Am.J.Cardiol. 6: 556-563.

Wilson JR, Lanoe V, Frey MJ, Ferraro LN (1988).
Effect on peripheral arterioles of chronic fluid and sodium retention in chronic heart failure. J.Am.Coll.Cardiol. 12: 202-208.

Wilson Jr, Schwartz JS, St.John Sutton M, Ferraro LN, Horowitz N, Reichel N, Josephsen ME (1983).
Prognosis in severe heart failure: relation to haemodynamic measurements and ventricular ectopic activity. J.Am.Coll.Cardiol. 3: 403-410.

Wilson JR, Wiener DH, Fink LI, Ferraro N (1986).
Vasodilatory behaviour of skeletal muscle arterioles in patients with non-oedematous chronic heart failure. Circulation 74: 775-779.

Wilson DM, Leutscher JA (1990).
Plasma prorenin and complications in children with insulin-dependent diabetes mellitus. N.Eng.J.Med. 323: 1101-1106.

Wright RS, Levine MS, Bellamy, PE et al (1990).
Ventilatory and diffusion abnormalities in potential heart transplant recipients. Chest 98: 816-820.

Xie G-Y, Berk MR, Smith MD, Demaria AN (1996).
Relation of Doppler transmitral flow patterns to functional status in congestive heart failure. Am. Heart J. 131: 766-771.

Yamaoka K, Nagawaka T, Uno T (1978).
Application of Akaike's Information Criterion (AIC) in the evaluation of linear pharmacokinetic equations. J.Pharmacokinet.Biopharmaceutic. 6: 165-175.

Yanagana N, Capparelli N, Jo OD, Friedal A, Barrett JD, Eggena P (1991).
Production of angiotensinogen and renin-like activity by rabbit proximal tubular cells in culture. Kidney Int. 39: 938-941.

Yatani A, Imoto Y, Scwartz, Brown AM. (1989).
A new positive inotropic agent OPC-8212 modulates Ca++ channels in ventricular myocytes of guinea-pig. J.Cardiovasc.Pharmacol. 13: 812-819

Yates DB (1991).
Pharmacology of flosequinan. Am.Heart J. 121: 974-983.

YeoWW, Foster G, Ramsay LE (1991).
Prevalence of persistent cough during long-term enalapril treatment: controlled study versus nifedipine. Q.J.Med. 81: 763-770.
YeoWW, Ramsay LE (1990).
Persistent dry cough with enalapril: incidence depends on method used.
J.Hum.Hypertens. 4: 517-520.

Yoshimura S, Imai K, Hayashi Y, Hashimoto K (1989).
Role of renin angiotensin system in the pathogenesis of spontaneous myocardial fibrosis in Sprague-Dawley rats: effect of long term administration of captopril. Tohoku J. Exp. Med. 157: 241-249.

Yusuf S, Peto R, Lewis J, Collins R, Sleight P. (1985).
Beta blockade during and after myocardial infarction: an overview of the randomised trials. Prog.Cardiovasc.Dis. 27: 335-371.

Yusuf S, Thom T, Abbott RD (1989).
Changes in hypertension treatment and in congestive failure mortality in the United States. Hypertension 13 (Suppl 1): 174-179.

Yusuf S, Garg R, Held P, Gorlin R (1992).
Need for a large randomised trial to evaluate the effects of digitalis on morbidity and mortality in congestive heart failure. Am.J.Cardiol. 69: 64G-70G.

Zatz R, Dunn BR, Meyer TW, Anderson S, Rennke HG, Brenner BM (1986).
Prevention of diabetic glomerulopathy by pharmacological amelioration of glomerular capillary hypertension. J.Clin.Invest. 77: 1925-1930.

Zatz R, Anderson S, Meyer TW, Dunn BR, Rennke HG, Brenner BM (1987).

Lowering of arterial blood pressure limits glomerular sclerosis in rats with with renal ablation and in experimental diabetes. Kidney Int. 31 (Suppl 20): S123-S129.

Zelis R, Flaim S (1982).
Alterations in vasomotor tone in congestive heart failure.
Prog.Cardiovasc.Dis. 24: 437-459.

Zelis R, Mason DT, Braunwald E (1968).
A comparison of the effects of vasodilator stimuli on peripheral resistance vessels in normal subjects and in patients with congestive heart failure. J.Clin.Invest. 47: 960-970.

Zeller K, Whittaker E, Sullivan L, Raskin P, Jacobson H (1991).
Effet of restricting dietary protein on the progression of renal failure in patients with insulin dependent diabetes mellitus. N.Engl.J.Med. 324: 78-84.

Ziesche S, Rector TS, Cohn JN (1995).
Interobserver discordance in the classification of mechanisms of death in studies of heart failure. J.Card.Failure 1: 127-132.

Zimmerman BG (1962).
Effect of acute sympathectomy on responses to angiotensin and norepineprine.
Proc.Soc.Exp.Biol.Med. 106: 832-846.

Appendix

Individual pharmacokinetic / pharmacodynamic parameters

Appendix 1.1
Infusion time and total dose given by constant rate intravenous infusion in Study 1.

A. Perindoprilat (proposed total dose of 1mg given by constant rate intravenous infusion over 6 hours)

Patient	Infusion duration (hours)	Total dose given (mg)
1	6	1
2	2.5	0.417
3	6	1
4	6	1
5	6	1
6	5	0.833
7	6	1
8	5	0.833
9	5	0.833
10	4	0.667
11	6	1
12	6	0.833

B. Enalaprilat (proposed total dose of 1.5mg given by constant rate intravenous infusion over 6 hours)

Patient	Infusion duration (hours)	Total dose given (mg)
1	6	1.5
2	6	1.5
3	6	1.5
4	6	1.5
5	2	0.5
6	2	0.5
7	6	1.5
8	6	1.5
9	3	0.75
10	4	1.0
11	6	1.5
12	5	1.25

Appendix 1.2

Pharmacokinetic parameters (1 compartment model) for enalaprilat given as constant rate intravenous infusion, 0.25mg / hr (718nmol / hr) for up to 6 hours.

Patient	A	Cl	V1	Ke	AUC	$t_{1/2}$	RSS	SIC	AIC	Time
1	0.229	4.36	19.0	0.229	988	3.02	3457	177	175	6
2	0.440	2.27	11.5	0.198	1893	3.50	23210	217	215	6
3	0.396	2.52	24.1	0.105	1706	6.63	4620	183	181	6
4	0.213	4.70	19.5	0.241	917	2.87	5774	188	186	6
5	0.211	4.74	20.8	0.228	303	3.04	1051	152	150	2
6	0.233	4.30	16.2	0.265	334	2.61	1168	154	152	2
7	0.139	7.21	29.6	0.243	597	2.85	1102	153	151	6
8	0.623	1.60	16.1	0.100	2683	6.95	7014	192	190	6
9	0.267	3.75	12.1	0.310	575	2.24	4430	182	180	3
10	0.170	5.89	22.3	0.265	487	2.62	1184	155	153	4
11	0.133	7.50	27.3	0.275	574	2.52	880	148	146	6
12	0.253	3.96	20.2	0.195	907	3.55	1519	160	158	5

where

A coefficient for exponential term
Ke Elimination rate constant (hr^{-1})
Cl Clearance (l / hr)
$t_{1/2}$ Elimination half-life (hours)
V Volume of distribution (l)
AUC Area under the plasma concentration time curve ($nmol.l^{-1}.hour$)
RSS Residual sum of squares
SIC Schwarz Information Criterion
AIC Akaike Information Criterion
Time Infusion duration (hours)

Appendix 1.3
Pharmacokinetic parameters (1 compartment model) for perindoprilat given as constant rate intravenous infusion, 0.167mg / hr (489nmol / hour) for up to 6 hours.

Patient	A	Ke	Cl	V	Cmax	$t_{1/2}$	AUC	RSS	AIC	SIC	Time
1	0.179	0.169	5.57	33.1	66	4.1	527	582	138	140	6
2	0.166	0.352	6.03	17.1	55	2	203	405	130	132	2.5
3	0.412	0.109	2.43	22.3	94	6.4	1006	1083	151	153	5
4	0.152	0.233	6.57	28.2	67	3	447	607	139	141	6
5	0.551	0.121	1.81	15.0	108	5.7	675	3310	174	176	2.5
6	0.080	0.233	12.5	53.6	32.3	3	235	227	118	120	6
7	0.016	0.292	9.4	32.1	47.9	2.4	312	445	132	134	6
8	0.277	0.214	3.62	16.9	117.5	3.2	810	2402	167	170	6
9	0.088	0.317	11.3	35.7	36.7	2.2	216	261-	121	123	5
10	0.155	1.1	6.45	58.6	38	0.6	303	336	126	128	4
11	0.162	0.178	6.18	34.7	56.4	3.9	475	142	108	110-	6
12	0.308	0.169	3.24	19.2	117.5	4.1	906	2357	167	169	6

where

A   coefficient for exponential term
Ke   Elimination rate constant (hr^{-1})
Cl   Clearance (l / hr)
V   Volume of distribution (l)
Cmax   Peak drug concentration (nmol/l)
$t_{1/2}$ Elimination half-life (hours)
AUC   Area under the plasma concentration time curve (nmol. l^{-1}.hour)
RSS   Residual sum of squares
SIC   Schwarz Information Criterion
AIC   Akaike Information Criterion
Time   Infusion duration (hours)

Appendix 1.4

Pharmacokinetic parameters (1 compartment model) for perindoprilat given as constant rate intravenous infusion, 0.167mg / hr (489nmol / hr) for 1 hour.

Patient	A	Ke	Cl	V1	Cmax	$t_{1/2}$	AUC	RSS	SIC	AIC
37	0.071	0.333	14.1	42.3	11.16	2.1	34.7	82.2	94.2	92.2
38	0.064	0.767	15.6	20.4	17.03	0.9	31.3	106	99.3	97.3
39					4.699					
40	0.131	0.376	7.64	20.3	26.73	1.8	64	204	112	110
41	0.0294	0.554	34.1	61.5	7.34	1.3	14.3	25.8	67.6	65.8
42	0.0303	0.289	32.9	114	4.699	2.4	14.9	16.3	61.8	59.9
43	0.0469	1.93	20.9	10.4	20.26	0.4	23.4	165	108	106
44	0.0758	0.108	13.2	122	4.405	6.4	37	6.22	40.6	38.7
45	0.0372	1.23	26.9	21.8	13.22	0.6	18.2	98	97.5	95.7
46	0.15	0.278	6.68	24	29	2.5	73.2	38.7	125	123
47	0.0266	0.922	37.6	40.8	8.22	0.8	13	38.9	79.2	77.2
48	0.0245	0.883	39.3	44.5	9.4	0.8	12.4	31.7	71.5	69.7

where
A   coefficient for exponential term
Ke   Elimination rate constant (hr^{-1})
Cl   Clearance (l / hr)
V   Volume of distribution (l)
Cmax   Peak drug concentration (nmol/l)
$t_{1/2}$ Elimination half-life (hours)
AUC   Area under the plasma concentration time curve ($nmol.l^{-1}.hour$])
RSS   Residual sum of squares
SIC   Schwarz Information Criterion
AIC   Akaike Information Criterion

Note:
Patient 39:   No fit - Cmax at 50 minutes (4.99u), 60mins = 4.7u, 75mins = .29u, 90mins = 2.64u, 105 mins = 1.45u, 120mins = 1.18u, 180mins = 4.7u

Appendix 1.5

Pharmacokinetic parameters (2 compartment model) for perindoprilat given as constant rate intravenous infusion, 0.167mg / hr (489nmol / hr) for 1 hour.

Patient	A x100	alpha	B	beta x100	AUC	$t_{1/2}$ alpha	$t_{1/2}$ beta	k10	Cl	Vdss	RSS	AIC	SIC
37	0.922	9.30	0.142	8.82	74	0074	7.86	0.65	6.61	70.4	16.3	63.8	67.8
38	1.70	4.12	0123	14.9	68.7	0.168	4.66	0.65	7.12	42.3	19.2	67.1	71.1
39													
40	5.33	0.929	1.12	0.987	572	0.746	70.2	0.052	0.855	82.7	121	104	108
41	0.668	5.45	0.093	6.73	48.6	0.127	10.3	0.429	10.1	140	4.27	35.6	39.4
42	0.451	4.26	0.060	7.06	31.7	0.163	9.82	0.362	15.4	204	5.36	41.6	45.6
43	2.79	4.75	0.180	7.36	102	0.146	9.41	0.7	4.8	56.6	22.5	70.3	74.3
44	0.396	2.00	0.211	2.56	105	0.346	27.1	0.062	4.64	178	1.62	17.2	21
45	1.92	2.78	0.380	2.08	195	0.249	33.3	0.153	2.5	-114	22.8	70.5	74.5
46	2.43	.997	0.169	14.1	94.6	0.695	4.92	0.249	5.17	32.7	368	126	130
47	0.968	3.72	0.149	4.29	77.6	0.186	16.2	0.267	6.3	138	2.8	28.6	32.6
48	0.541	10.1	0.058	16.6	30.8	0.068	4.19	1.02	15.9	87.8	13.5	57.4	61.2

where

A, B	Coefficients for exponential term
alpha, beta	Macroscopic rate contstants (hr^{-1})
$t_{1/2}$ -alpha	Half life of drug during distribution phase
$t_{1/2}$ -beta	Half life of drug during elimination phase
Cl	Clearance (l / hr)
Vdss	Volume of distribution at steady state (l)
Time	Infusion duration (hours)
AUC	Area under the plasma concentration time curve ($nmol.l^{-1}.hour$) (AUC = Dose / Cl)
k10	Elimination rate constant from compartment 1 to 0

237

Appendix 2.1
Pharmacokinetic parameters for enalaprilat (1 compartment, zero order absorption) given as a
single dose of enalapril 2.5mg p.o.

Patient	A	Ke	Tabs	Tlag	RSS	AIC	SIC	Cmax	AUC	$t_{1/2}$	Cl/F	V/F
13	0.0265	0.3610	4.19	1.820	183.00	117.0	122.0	33.010	136	1.92	37.6912	104.408
14	0.0697	0.0862	3.79	0.626	42.20	86.6	90.8	24.400	357	8.04	14.3585	166.572
15	0.0712	0.1560	5.19	1.700	27.90	77.9	82.1	34.440	365	4.44	14.0438	90.025
16	0.3510	0.0432	4.77	1.220	307.00	128.0	132.0	71.760	1798	16.00	2.8509	65.994
17	0.1250	0.0665	4.72	0.435	66.80	96.2	100.0	39.040	641	10.40	7.9969	120.254
18	0.0840	0.0650	9.70	0.302	121.00	109.0	113.0	17.220	430	-10.70	11.9209	183.399
19	0.0952	0.0458	7.71	1.600	10.80	57.9	62.1	18.940	488	15.10	10.5041	229.347
20	0.1150	0.0821	5.51	1.330	13.30	62.4	66.6	34.440	588	8.45	8.7177	106.184
21	0.1280	0.0857	4.32	1.970	52.70	91.2	95.4	44.490	656	8.08	7.8140	91.179
22	0.0749	0.0172	2.57	0.657	4.94	41.5	45.5	6.315	384	40.30	13.3490	776.102
23	0.0781	0.1100	3.88	0.623	4.48	39.5	43.7	34.440	400	6.31	12.8150	116.500
24	0.0811	0.0856	4.78	1.180	17.70	68.3	72.5	28.700	415	8.10	12.3518	144.297
25	0.1220	0.0656	6.38	0.791	21.00	72.0	72.6	32.430	627	10.60	8.1754	124.626
26	0.1140	0.0826	4.02	0.779	42.80	86.9	91.0	38.460	585	8.39	8.7624	106.082
27	0.0645	0.1000	3.33	1.220	177.00	117.0	121.0	26.690	330	6.91	15.5333	155.333
28	0.0622	0.0838	4.46	1.140	23.60	74.4	78.5	21.810	319	8.26	16.0690	191.754
29	0.1450	0.0492	5.31	1.200	21.10	72.0	76.2	31.290	744	14.10	6.8898	140.036
30	0.0681	0.0876	3.28	2.450	2.82	29.8	34.0	25.830	349	7.91	14.6877	167.668
31	0.6340	0.0208	4.79	0.878	67.50	96.5	101.0	65.440	3252	33.20	1.5763	75.782
32	0.0380	0.0910	7.32	1.920	19.20	70.0	74.2	12.060	195	7.61	26.2872	288.870
33	0.0839	0.1180	4.05	0.323	168.00	116.0	120.0	38.170	430	5.88	11.9209	101.025
34	0.1080	0.0884	5.55	1.810	49.70	90.0	94.2	34.440	554	7.84	9.2527	104.669
35	0.0402	0.0271	8.72	1.660	1.31	13.7	17.9	4.880	206	25.50	24.8835	918.210
36	0.1730	0.0730	6.61	1.080	275.00	126.0	130.0	53.390	888	9.49	5.7725	79.076

Abbreviations as per previous Tables.

Appendix 2.2
Pharmacokinetic parameters for enalaprilat (1 compartment, 1st order absorption) given as single dose of enalapril 2.5mg p.o.

Patient	A	Ke	Ka	Tlag	RSS	AIC	SIC	Cmax
13	147	0.322	0.478	2.40	347	131	135	33.01
14	92.4	0.0963	0.48	0.954	10.8	57.9	62.1	24.4
15	169	0.180	0.305	2.28	71	97.5	102	34.44
16	1570	0.123	0.139	1.46	39.1	85	89.2	71.76
17	120	0.111	0.238	0.862	170	116	120	39.04
18	478	0.0972	0.109	1.03	179	117	121	17.22
19	195	0.0924	0.117	0.695	13.9	63.3	67.5	18.94
20	728	0.145	0.164	1.62	27.9	77.9	82.1	34.44
21	141	0.130	0.296	2.38	84.4	101	105	44.49
22	7.6	0.0201	0.794	0.925	2.57	27.8	32	6.315
23	141	0.175	0.317	0.934	63.9	95.3	99.5	34.44
24	827	0.166	0.180	1.44	56.5	92.7	96.9	28.7
25	557	0.119	0.138	1.18	77.5	99.4	104	32.43
26	169	0.152	0.276	1.11	93.2	103	107	38.46
27	549	0.136	0.517	1.63	197	119	123	26.69
28	69.0	124	0.271	1.43	54.6	92	96.2	21.81
29	52.6	0.0586	0.301	1.53	17.9	68.6	72.8	31.29
30	43.1	0.104	0.538	2.75	20.9	71.9	76.1	25.83
31	94.7	0.0346	0.303	1.26	199	119	123	65.44
32	115	0.134	0.175	2.83	17.2	67.7	71.9	12.06
33	121	0.159	0.343	0.585	207	120	124	38.17
34	718	0.154	0.176	2.37	59.4	93.8	98	34.44
35	314	33.8	0.0737	0.107	2.5	1.65	18.5	4.88
36	597	0.121	0.149	1.54	276	126	130	58.39

where
A   coefficient for exponential term
Ke   elimination rate constant (hr^{-1})
Ka   Absorption rate constant
Tlag   Lag phase (absorption)
RSS   Residual sum of squares
AIC   Akaike information criterion
SIC   Schwarz information criterion

where
A= coefficient for exponential term; Ke= elimination rate constant in hr^{-1}
Ka= absorption rate constant in hr^{-1}; Tlag=Lag phase time in hours
AUC=Area under the plasma concentration time curve in nmol / (l/ hour)
Other abbreviations as per previous Tables.

Appendix 3.1

Pharmacokinetic parameters for quinaprilat (1 compartment, zero order absorption) givena s a single dose of quinapril 2.5mg p.o.

Patient	A	Ke	Tabs	Tlag	RSS	AIC	SIC	AUC	$t_{1/2}$	Cmax	Cl/F	V/F
1	0.2120	0.168	2.53	0.890	244	123	128	1210	4.11	149.30	4.7107	28.0401
2	0.1120	0.202	1.31	1.380	241	123	127	641	3.43	103.80	8.8924	44.0216
3	0.2520	0.252	1.59	1.920	734	147	151	1435	2.75	252.10	3.9721	15.7624
4	0.1080	0.189	2.36	1.300	263	125	129	617	3.68	86.24	9.2382	48.8796
5	0.1170	0.167	1.31	0.406	759	147	151	669	4.14	94.76	8.5202	51.0190
6	0.6540	0.061	2.16	0.315	2874	175	179	3726	11.4	197.80	1.5298	25.0785
7	0.0638	0.371	1.99	1.420	321	129	133	364	1.87	83.07	15.6593	42.2085
8	0.0907	0.269	1.88	0.788	714	146	150	517	2.58	100.60	11.0251	40.9857
9	0.1390	0.164	1.19	2.810	1000	153	157	793	4.23	123.50	7.1879	43.8286
10	0.0907	0.272	4.95	0.571	863	150	154	517	2.55	72.11	11.0251	40.5336
11	0.1850	0.184	2.71	1.010	854	143	147	1054	3.77	157.40	5.4080	29.3911
12	0.0655	0.279	3.24	0.899	282	121	125	373	2.48	71.62	15.2815	54.7724

A   Coefficient for exponential term
Ke   Elimination rate constant (hr^{-1})
Tabs   Absorption time (hours)
Tlag   Lag time (hours)
RSS   residual sum of squares
SIC   Schwartz Information criterion
AIC   Akaike Information Criterion
AUC   Area under plasma concentration time curve (nmol/[l/hour])
Cmax   Peak plasma drug concentration
Cl   Clearance (l/hr)
F   Bioavailability

Appendix 3.2

Pharmacokinetic parameters for quinaprilat (1 compartment, first order absorption) given as a single dose of quinapril 2.5mg p.o.

Patient	A	Ke	Ka	Tlag	RSS	AIC	SIC	AUC	$t_{1/2}$	Cmax
1	496	0.253	0.562	1.140	467	137	141	1078	2.74	149.30
2	202	0.260	1.08	1.470	421	135	139	588	2.66	103.80
3	1750	0.433	0.639	1.950	109	107	111	1301	1.60	252.10
4	230	0.262	0.721	1.600	412	134	139	559	2.65	86.24
5	175	0.234	1.190	0.580	797	148	152	602	2.96	94.76
6	281	0.0779	0.765	0.537	3228	178	182	3243	-8.90	197.80
7	304	0.450	0.943	1.660	602	142	147	354	1.54	83.07
8	210	0.308	1.110	1.120	1187	157	161	491	2.25	100.60
9	145	0.174	9.570	2.980	981	153	157	820	3.99	123.50
10	1390	0.286	0.325	0.933	755	147	151	579	2.42	72.11
11	374	0.234	0.626	1.310	1964	160	164	1000	2.96	157.40
12	9390	0.408	0.415	1.180	629	137	141	377	1.70	71.62

A   Coefficient for exponential term
Ke   Elimination rate constant (hr^{-1})
Ka   Absorption rate constant (hr^{-1})
Tlag Lag time (hours)
RSS   residual sum of squares
SIC   Schwartz Information criterion
AIC   Akaike Information Criterion
AUC   Area under plasma concentration time curve (nmol/[l/hour])
Cmax Peak plasma drug concentration
Cl   Clearance (l/hr)
F   Bioavailability

Appendix 4.1
Pharmacokinetic parameters (1 compartment, zero order absorption) for perindoprilat
given as single dose of perindopril 2mg p.o.

Patient	A	Ke	Tabs	Tlag	RSS	AIC	SIC	Cmax	AUC	Cl/F	V/F
13								8.5			
14								21.15			
15	0.0824	0.165	1.46	0.577	172	116	120	32.31	224	12.1	73.4
16	0.0895	0.0640	2.81	0.809	7.11	49.2	53.4	14.68	243	11.2	1755
17								11.16			
18	0.134	0.0553	6.54	0.228	42.3	86.7	90.8	17.62	365	7.4	134
19	0.121	0.0373	.510	0.625	13.2	62.2	66.4	12.34	329	8.2	221
20	0.0889	0.0416	1.08	0.661	9.63	55.6	59.7	9.98	241	11.3	271
21	0.106	0.0281	4.11	1.34	5.08	42.1	46.3	7.34	287	9.5	337
22								7.34			
23								17.62			
24	0.117	0.0229	2.89	0.963	2.53	27.5	31.7	7.34	319	8.51	372
25								10.28			
26	0.0417	0.205	4.44	0.27	54.8	88.1	92	12.92	113	24.0	117
27								10.87			
28	0.089	0.0521	3.71	0.549	7.2	47.5	51.5	10.57	241	11.3	216
29	0.0946	0.0315	3.53	0.52	3.43	32.7	36.6	8.57	257	10.6	335
30								6.17			
31								22.61			
32	0.0604	0.0418	.384	1.19	8.42	48.5	52.3	7.93	164	16.5	396
33	0.0358	0.0126	1.03	0.782	21.5	69.4	73.7	12.63	97.3	27.9	2214
34	0.169	0.0519	4.18	0.832	43.7	83.5	87.5	20.26	458	5.9	114
35								12.04			
36								10.0			

13	No fit - concs still increasing at 10 hours - absn not 0 order
14	No fit - double peak at 3h (12u) and 8h (21u)
17	No fit - reached 9.4u by 4h then all concs > 8u
19	No fit - double peaks at 1.75h (12.34u) and 6h (12.34u)
20	No fit - double peaks at 2-3h (10u) and 6h (8.7u)
22	No fit - double peaks at 6h (7u) and 10h (7.3u)
23	No fit - double peaks at 6h (17.6u) and 10h (17u)
25	No fit - double peaks at 4h (9.7u) and 8h (10.3u)
27	No fit - double peaks at 4h (9.7u) and 8h (10.3u)
30	No fit - double peaks at 4-5h (6.2u) and 8h (5.6u)
31	No fit - still in absorption phase at 10h
34	1st order absorption may be better
35	No fit - double peaks at 3h (9.7u) and 6h (12.04u)
36	No fit - double peaks at .83 (10u) and 4h (5u)

where
A= coefficient for exponential term; Ke= elimination rate constant in hr^{-1}
Tabs=Absorption time in hours; Tlag=Lag phase time in hours
AUC=Area under the plasma concentration time curve in nmol / (l/ hour)
Cl/F=Clearance/Bioavailability; V/F=Volume of distribution /Bioavailability
Other abreviations as per previous Tables.

Appendix 4.2

Pharmacokinetic parameters for perindoprilat (1 compartment, 1st order absorption) given as single dose of perindopril 2mg p.o.

Patient	A	Ke	Ka	Tlag	RSS	AIC	SIC	Cmax	AUC	$t_{1/2}$
13								8.5		
14								21.15		
15	43.5	0.167	1.52	0.762	232	122	127	32.31	218	3.93
16	17.2	0.0647	0.795	1.17	12.3	60.7	64.9	14.68	244	10.7
17								11.16		
18	47.3	0.0769	0.183	0.550	49.4	89.9	94.1	17.62	357	9.02
19	12.8	0.0413	3.47	0.658	12.2	60.6	64.8	12.34	307	16.8
20	10.7	0.0469	2.04	0.800	9.49	55.3	59.4	9.98	223	14.8
21	9.34	0.0335	0.511	1.85	2.68	28.7	32.9	7.34	261	20.7
22								7.34		
23								17.62		
24	7.86	0.0252	0.716	1.25	2.52	27.4	31.5	7.34	301	27.5
25								10.28		
26	146	0.264	0.335	0.637	38.9	81.2	85.2	12.92	117	2.63
27								10.87		
28	16.6	0.0640	0.459	0.833	1.01	8.13	12.1	10.57	224	10.8
29	9.34	0.0362	0.532	0.868	9.3	52.6	56.6	8.57	241	19.2
30					.			6.17		.
31								22.61		
32	6.98	0.0433	6.21	1.22	8.81	49.3	53.1	7.93	160	16
33	13.9	0.145	2.41	0.975	32.5	77.6	81.6	12.63	90.2	4.79
34	40.3	0.0778	0.336	1.14	10.5	55.1	59.1	20.26	398	8.91
35								12.04		
36								10.0		

13   No fit - concs still increasing at 10 hours - absn not 0 order
14   No fit - double peak at 3h (12u) and 8h (21u)
17   No fit - reached 9.4u by 4h then all concs > 8u
19   No fit - double peaks at 1.75h (12.34u) and 6h (12.34u)
20   No fit - double peaks at 2-3h (10u) and 6h (8.7u)
22   No fit - double peaks at 6h (7u) and 10h (7.3u)
23   No fit - double peaks at 6h (17.6u) and 10h (17u)
25   No fit - double peaks at 4h (9.7u) and 8h (10.3u)
27   No fit - double peaks at 4h (9.7u) and 8h (10.3u)
30   No fit - double peaks at 4-5h (6.2u) and 8h (5.6u)
31   No fit - still in absorption phase at 10h
34   1st order absorption may be better
35   No fit - double peaks at 3h (9.7u) and 6h (12.04u)
36   No fit - double peaks at .83 (10u) and 4h (5u)

where
A= coefficient for exponential term; Ke= elimination rate constant in hr^{-1}
Ka= absorption rate constant in hr^{-1}; Tlag=Lag phase time in hours
AUC=Area under the plasma concentration time curve in nmol / (l/ hour)
Other abbreviations as per previous Tables.

Appendix 5.1

Estimated individual β-coefficient and intercept of regression line relating fall in MAP to plasma drug concentration.

β-coefficient (mm Hg.nmol^{-1}.l)

Patient	Quinaprilat Oral Study 3	Enalaprilat I.V. Study 1	Enalaprilat Oral Study 1	Enalaprilat Oral Study 2	Perindorilat I.V.Study 1	Perindoprilat Oral Study 1	Perindoprilat Oral Study 2	Perindoprilat I.V. Study2
1	-0.02	0.04	-1.03	-0.64	0.00	-1.78	-0.80	-0.88
2	-0.10	-0.05	-0.14	-0.82	-0.47	-0.22	-1.14	0.03
3	-0.06	-0.03	-0.23	-0.40	-0.11	0.08	-0.53	0.60
4	-0.10	-0.15	-0.14	-0.26	-0.39	-0.91	-1.52	-0.48
5	-0.01	-0.34	-0.22	-0.56	-0.20	0.85	-1.80	-1.59
6	0.03	-0.25	-0.62	-0.31	-0.32	-1.10	-0.70	-1.75
7	0.03	-0.24	-1.33	-0.19	-0.1	-0.25	0.07	-0.86
8	0.01	-0.09	-0.28	-0.45	-0.13	0.57	0.78	-1.21
9	-0.13	-0.17	-0.4	-0.23	-0.46	0.46	1.21	-0.61
10	-0.06	-0.46	-0.31	-0.62	-0.67	1.02	-0.77	-0.06
11	0.03	-0.05	-0.52	0.92	-0.07	-0.83	-0.16	-0.60
12	-0.06	-0.15	-0.35	-0.48	-0.15	-0.12	0.31	-0.50

Intercept (mm Hg)

Patient	Quinaprilat Oral Study 3	Enalaprilat I.V. Study 1	Enalaprilat Oral Study 1	Enalaprilat Oral Study 2	Perindoprilat I.V.Study 1	Perindoprilat Oral Study 1	Perindoprilat Oral Study 2	Perindoprilat I.V. Study2
1	-1.43	-2.68	-9.73	4.13	1.95	5.96	4.2	1.38
2	-2.31	-2.5	-1.96	7.02	0	-6.24	11.05	-4.07
3	-4.26	-0.34	-6.42	2.12	-6.3	-2.31	9.57	1
4	-2.39	5.5	-4.24	10.04	7.68	0.05	12.21	-0.26
5	-9.86	4.56	-5.13	-1.75	-9.54	-8.2	2.04	10.2
6	-2.21	1.8	-0.15	3.84	3.62	10.56	12.45	0.75
7	-1.46	-5.07	-6.15	1.26	1.83	2.11	-2.11	-2.88
8	-1.73	-13.63	-1.47	1.07	5.4	-5.41	-2.41	-3.83
9	-2.36	-2.34	-5.29	-6.76	-3.1	8.6	2.45	6.9
10	-7.3	3.88	0.95	2.97	6.85	4.91	4.47	-6.49
11	9.77	0.06	3.43	1.37	-2.4	0.44	4.97	4.53
12	-2.79	-12.87	-8.07	2.27	1.44	-3	-8.17	4.24

Appendix 6.1
Demographic data for patients receiving enalaprilat

Patients 1-12=0.25mg/hr i.v. enalaprilat for 6 hours; 13-36=2.5mg oral enalapril 2.5mg

Patient	Study	Age	Sex	Aetiology	NYHA	Cardiac Rhythm	Na^+	K^+	Creatinine	Initial MAP	Initial ACE	Initial PRA
1	1	72	2	1	3	1	146	3.6	87	102	17.9	0.09
2	1	71	2	2+3	3	1	140	3.6	94	121	31.4	0.28
3	1	74	2	2	3	2	140	4.5	101	116	27.2	0.97
4	1	60	1	3	3	2	141	4.7	117	79	19.9	3.16
5	1	73	1	1	3	2	137	3.7	182	80	24.3	4.53
6	1	76	2	2	3	2	140	3.9	82	75	13.9	5.65
7	1	60	1	3+4	2	1	138	4.4	117	99	28.9	12.24
8	1	77	2	4	3	1	143	3.5	132	112	25.6	9.09
9	1	77	2	2	3	2	143	3.3	76	91	16.8	4.28
10	1	70	1	2+3	3	2	139	4.5	97	110	19.4	5.79
11	1	66	1	2	2	2	140	4.0	108	102	22.2	1.50
12	1	63	1	3+4	3	2	140	4.2	106	102	22.9	2.23
13	1	63	2	2+1	2	1	141	4.1	85_	133	26	0.74
14	1	63	1	2	3	1	142	3.8	107	101	46	1.66
15	1	82	2	2	3	1	139	4.9	84	94	17	1.80
16	1	66	2	2	3	1	138	3.0	180	116	16	6.05
17	1	58	1	3	3	1	145	4.3	111	107	20	0.47
18	1	78	1	3	3	1	136	4.1	77	97	20	1.12
19	1	60	2	1+3	3	1	137	5.2	93	120	23	45.00
20	1	60	1	2+3	3	5	139	3.6	120	101	22	7.53
21	1	66	1	2	3	1	141	4.6	117	91	20	
22	1	62	1	2	2	1	142	4.8	75	112	15	0.52
23	1	64	2	2	2	1	140	3.7	83	99	29	0.69
24	1	69	2	3	2	1	144	3.8	89	103	21	0.47
25	2	75	2	2+4	3	1	135	4.1	89	76	17.5	25.34
26	2	75	1	2	2	2	141	4.1	120	92	19.6	3.64
27	2	70	1	2+4	2	1	141	4.7	113	100	19.2	0.75
28	2	55	1	2	3	2	138	4.4	118	74	8.9	4.03
29	2	71	1	1	4	1	135	4.0	121	86	35.7	14.64
30	2	76	1	2	2	2	139	4.1	122	83	27.9	3.71
31	2	66	2	2	4	2	125	3.3	177	69	30.5	33.30
32	2	72	1	2	2	2	139	4.1	100	80	17.9	3.36
33	2	66	1	2	2	2	130	3.4	101	102	26.4	
34	2	69	2	2	2	2	143	4.9	126	104	22.8	9.44
35	2	75	1	2	3	2	135	4.3	98	83	22.4	3.19
36	2	77	1	2	3	1	138	4.7	192	106	27.7	13.30

		Aetiology (Sum of all present)	Cardiac rhythm (Sum of all present)
Age	years		
Sex	1=Male; 2=Female	1 Alcohol related cardiomyopathy	1 Atrial fibrillation
Na^-	mmol/L	2 Ischaemic heart disease	2 Sinus rhythm
K^-	mmol/L	3 Hypertensive heart disease	4 Sinus rhythm – Frequent ventricular
Cr	µmol/L	4 Valvular heart disease	premature complexes
MAP	mm Hg		
PRA	Plasma Renin activity in ng A1 ml/hr		
ACE	Angiotensin Converting Enzyme activity (ng Ang I/ml/Hour)		

Appendix 6.2

Demographic data for patients receiving perindoprilat. Patients1-12=0.167mg/hr iv 6hr; 13-36=2mg oral; 37-48=0.167mg/hr iv 1hr

Patient	Study	Age	Sex	Aetiology	NYHA	Cardiac Rhythm	Na$^+$	K$^+$	Creatinine	Initial MAP	Initial ACE	Initial PRA
1	1	64	1	3	3	2	143	4.5	130	96	15.3	0.72
2	1	84	2	2	2	1	132	4.3	83	97	23.5	2.11
3	1	87	2	2	3	1	145	4.6	122	92	22.7	0.35
4	1	62	2	2	3	1	140	4.6	68	83	29.7	0.96
5	1	65	1	2	2	2	140	4.4	80	100	14.1	0.33
6	1	68	1	2	2	2	141	3.7	129	110	26.2	1.96
7	1	64	1	2+3	2	2	138	4.6	87	80	13.9	0.54
8	1	71	2	2+3	3	2	139	4.8	118	83	15.6	13.05
9	1	63	1	2+3	3	2	136	4.6	83	78	26.9	10.2
10	1	60	2	3	3	2	140	3.9	93	87	19.8	9.77
11	1	71	1	3+2	2	2	141	3.6	81	80	12.5	8.7
12	1	73	1	3+2	3	4	140	3.8	142	83	29.8	5.7
13	1	59	1	2+3	3	4	136	3.9	121	118	29	1.4
14	1	72	2	3	2	1	140	3.6	62	83	12	10.93
15	1	85	2	1+3	3	1	141	5.2	111	87	33	4.76
16	1	74	2	2	3	2	140	3.9	89	98	18	1.21
17	1	66	1	2+3	3	2	140	3.5	124	113	24	4.3
18	1	64	2	2	2	2	143	4.4	87	102	37	1.4
19	1	60	1	2	3	2	143	3	159	96	20	11.9
20	1	69	1	3	3	1	144	3.6	143	84	20	2.15
21	1	73	1	2+3	3	2	143	3.8	169	98	21	0.2
22	1	61	1	2	3	2	138	4	179	88	26	10.2
23	1	79	2	3	3	2	143	3.1	117	101	17	3.07
24	1	69	2	3+2	2	2	139	4.4	98	111	29	0.79
25	2	71	1	2	3	2	136	5.3	162	85	14.8	1.49
26	2	66	1	2	3	1	140	4	118	84	20.1	3.8
27	2	79	2	2+3	2	1	141	4.1	114	85	17	2.77
28	2	75	1	2	2	2	145	4.2	112	94	17	3.12
29	2	78	1	2	3	1	143	4.4	83	95	24.5	1.63
30	2	69	1	1	3	1	135	4.4	99	103	32	0.66
31	2	73	2	2	3	2	135	4.7	287	93	18.9	2.81
32	2	52	1	2+1	3	2	139	4	102	75	34.3	3.14
33	2	56	1	2	2	2	145	4.8	101	101	18.6	9.25
34	2	71	2	2	2	2	135	4.2	99	102	25.7	34.24
35	2	64	1	2	2	2	136	4.7	96	82	34	2.22
36	2	81	2	2	3	2	141	3.5	123	76	24.4	8.87
37	2	67	2	4+3	2	1	140	4.3	97	108	33.5	4.81
38	2	54	2	2	2	2	141	4.2	87	71	17.4	2.24
39	2	62	1	2	3	2	137	4.8	94	82	10	2.96
40	2	61	2	2	3	2	137	3.2	107	84	21.9	27.7
41	2	76	1	2	3	1	144	4.4	115	110	15.6	0.34
42	2	53	1	1	3	1	139	3.9	140	83	29.2	6.83
43	2	77	1	2	3	1	134	3.3	131	94	35.8	19.6
44	2	69	1	2	2	2	141	5	172	102	16.2	2.76
45	2	71	1	2	3	1	136	4.1	127	87	30	4.58
46	2	69	1	2	3	2	144	4.9	109	80	25.6	0.62
47	2	71	1	2	3	2	133	4.1	140	95	18	1.45
48	2	60	1	2	2	2	135	3.3	121	92	21	8.21

Abbreviations as per Appendix 6.1

Appendix 6.3
Demographic data for patients receiving quinaprilat.
Patients 1-12=oral quinapril 2.5mg

Patient	Study	Age	Sex	Aetiology	NYHA	Cardiac Rhythm	Na$^+$	K$^+$	Creatinine	Initial MAP	Initial ACE	Initial PRA
1	1	79	2	3+2	3	1	146	3.6	87	102	17.9	0.09
2	1	67	1	2	3	1	140	3.6	94	121	31.4	0.28
3	1	88	2	2	3	2	140	4.5	101	116	27.2	0.97
4	1	81	2	3	3	1	141	4.7	117	79	19.9	3.16
5	1	75	1	2	3	2	137	3.7	182	80	24.3	4.53
6	1	85	2	2+3	3	2	140	3.9	82	75	13.9	5.65
7	1	68	2	3	2	2	138	4.4	117	99	28.9	12.24
8	1	61	2	2+3	3	2	143	3.5	132	112	25.6	9.09
9	1	76	1	2	3	2	143	3.3	76	91	16.8	4.28
10	1	74	1	2	3	1	139	4.5	97	110	19.4	5.79
11	1	83	2	2	2	1	140	4.0	108	102	22.2	1.50
12	1	72	1	24	3	1	140	4.2	106	102	22.9	2.23

		Aetiology (Sum of all present)	Cardiac rhythm (Sum of all present)
Age	years	1 Alcohol related cardiomyopathy	1 Atrial fibrillation
Sex	1=Male; 2=Female	2 Ischaemic heart disease	2 Sinus rhythm
Na$^+$	mmol/L	3 Hypertensive heart disease	4 Sinus rhythm + Frequent ventricular
K$^+$	mmol/L	4 Valvular heart disease	premature complexes ·
Cr	μmol/L		
MAP	mm Hg		
PRA	Plasma Renin activity in ng A1 ml/hr		
ACE	Angiotensin Converting Enzyme activity (ng Ang I/ml/Hour)		

Appendix 7.1

Spearman rank correlation coefficient for the relationship between β-coefficient of regression line relating fall in MAP to plasma drug concentration, and baseline continuous variables.

Drug	Form	Study	Na$^+$	K$^+$	Cr	Initial MAP	Initial ACE	Initial PRA	Age	Diuretic dose
Quinaprilat	oral	3	-0.653	-0.452	-0.168	0.074	0.025	0.009	-0.021	-0.190
Enalaprilat	i.v.	1	0.607	-0.144	-0.102	0.493	0.231	-0.755	0.035	
Enalaprilat	oral	1	0.355	-0.34	0.441	-0.133	-0.268	-0.024	-0.032	
Enalaprilat	oral	2	-0.524	-0.225	-0.084	-0.322	0.168	-0.193	-0.349	
Enalaprilat	oral/i.v.	1+2	0.183	-0.337	0.046	0.064	0.036	-0.294	-0.020	-0.078
Perindoprilat	i.v.	1	0.513	0.085	0.298	-0.166	-0.573	-0.329	0.298	
Perindoprilat	oral	1	0.036	-0.046	0.573	-0.371	-0.011	0.228	0.088	
Perindoprilat	oral	2	-0.074	-0.011	0.165	-0.312	0.235	0.364	-0.354	
Perindoprilat	i.v.	2	-0.116	0.021	-0.651	-0.685	-0.273	-0.049	-0.26	
Perindoprilat	oral/i.v.	1+2	0.122	0.082	0.078	-0.354	-0.098	0.050 -	-0.145	0.212
All treatments	oral/i.v.	1+2+3	0.041	0.160	-0.070	0.068	-0.143	-0.009	-0.112	0.019

Age   years
Sex   1=Male; 2=Female
Na$^+$ mmol/L
K$^+$ mmol/L
Cr   μmol/L
MAP   mm Hg
PRA   Plasma Renin activity in ng A1 ml/hr
ACE   Angiotensin Converting Enzyme activity
           (ng Ang I/ml/Hour)

Appendix 7.2

Spearman rank correlation coefficient for relationship between AUC10 (Area under mean arterial pressure curve to 10 hours after initiation of therapy) and baseline continuous variables.

Drug	Form	Study	Na$^+$	K$^+$	Cr	Initial MAP	Initial ACE	Initial PRA	Age	Diuretic dose
Quinaprilat	oral	3	-0.494	-0.549	-0.266	-0.011	0.231	-0.017	-0.21	-0.052
Enalaprilat	i.v.	1	0.102	0.081	-0.126	-0.162	-0.175	-0.643	-0.021	
Enalaprilat	oral	1	0.158	-0.253	-0.238	-0.284	-0.07	-0.138	-0.176	
Enalaprilat	oral	2	0.028	0.271	-0.294	-0.588	-0.42	-0.382	-0.109	
Enalaprilat	oral/i.v	1+2	-0.042	0.014	-0.005	-0.380	-0.200	-0.276	-0.010	-0.305
Perindoprilat	i.v.	1	0.271	-0.157	0.441	-0.085	-0.294	0.028	-0.07	
Perindoprilat	oral	1	0.176	0.475	0.524	-0.207	0.589	-0.126	-0.161	
Perindoprilat	oral	2	0.028	0.197	-0.021	0.004	-0.014	-0.098	-0.469	
Perindoprilat	i.v.	2	-0.134	-0.011	0.112	0.462	-0.28	-0.315	0.063	
Perindoprilat	oral/i.v.	1+2	0.011	0.074	0.370	0.115	0.093	-0.090	-0.188	-0.051
All treatments	oral/i.v.	1+2+3	-0.125	-0.038	0.009	0.158	-0.177	-0.025	-0.123	-0.164

Age   years
Sex   1=Male; 2=Female
Na$^-$ mmol/L
K$^+$ mmol/L
Cr   μmol/L
MAP   mm Hg
PRA   Plasma Renin activity in ng A1 ml/hr
ACE   Angiotensin Converting Enzyme activity
            (ng Ang I/ml/Hour)