CHAPTER 1

INTRODUCTION

1.1 ANGIOTENSIN-CONVERTING ENZYME (ACE) INHIBITORS

In 1965, Ferreira demonstrated that the venom of the poisonous Brazillian pit viper Bothrops jararaca contained a potent inhibitor of bradykininase, an enzyme responsible for the breakdown of bradykinin. This enzyme later became known as angiotensin-converting enzyme or ACE (Ferreira SH *et al.* 1969). No specific ACE inhibitors were known until the discovery of teprotide (William & Hollenberg 1977; Curtiss *et al.*1978) and captopril (Cushman *et al.*1978; Ondetti *et al.*1977). Although teprotide was actually the first ACE inhibitor to be found, its cost and the need for intravenous administration limited its clinical evaluation. With the discovery of the orally active agent captopril came the opportunity to effectively block the renin-angiotensin-aldosterone system (RAAS).

There have been an increase in the usage of angiotensin-converting enzyme (ACE) inhibitors to treat hypertension and congestive heart failure over the past ten years. Enthusiasm for this class of agents is mainly related to their therapeutic efficacy and the paucity of significant adverse effects. Since captopril was characterised in the late 1970's, over 50 molecules with ACE inhibitory activity with the potential for clinical application have been synthesised. Today, out of fifteen ACE inhibitors which has gone through clinical development, only 10 oral compounds are approved by the FDA (V.G.Catherine, 1997).

Individual ACE inhibitors are different from each other in pharmacological features such as the presence or absence of a sulphydryl (SH) group, their lipid solubility, plasma half-life, route of excretion, whether prodrug or not, their oral and tissue bioavailability and by their

affinity for binding to ACE. Hypertension and congestive heart failure are the two major, accepted indications for the use of ACE inhibitors. ACE inhibitors are excellent tools to control blood pressure in patients with essential hypertension.

1.1.1 Mechanism of action

Angiotensin-converting enzyme (ACE) inhibitors are an important therapeutic advance in the treatment of patients with hypertension and congestive heart failure. In addition, they are useful pharmacological probes to assess the contribution of the renin–angiotensin system to circulatory homeostasis.

Traditionally, the renin-angiotensin system was regarded as a classical endocrine system. Recent discoveries for the last five years concerning the molecular biology of this system revealed that angiotensin II is synthesised not only in the circulation but also locally in tissues. The same holds true for bradykinin and related kinins (Linz W. *et al.* 1995). ACE is widely distributed in different tissues and cells, particularly on the luminal surface of the vascular endothelium. It is also found in epithelial cells in the kidney, gastrointestinal tract, testis and bodily fluids such as plasma and cerebrospinal fluids (Catherine VG. 1997).

Figure 1.1 shows the flow and site of action of ACE inhibitors (adapted from Katzung, 1989). Renin is a protease secreted by granular juxtaglomerular cells within the wall of the afferent arterioles of the renal glomeruli. This glycoprotein, which has a half-life of about 30 – 60 minutes, catalyses the generation of the decapeptide angiotensin I from a hepatic synthesised substrate angiotensinogen. Angiotensin I, which is largely vasoinactive, is converted to an octapeptide angiotensin II in the presence of the pulmonary and nonpulmonary tissue-bound ACE. Angiotensin II is a potent vasoconstrictor that increases vascular resistance, hence

increasing afterload and the blood pressure. Angiotensin II also directly stimulates aldosterone secretion from the adrenal cortex, which increases sodium and water reabsorption from the distal tubules.

ACE is also known as kininase II, the enzyme that degrades bradykinin (Unger T.1994; Linz W. 1995). Kinin exerts vasoactive effects by releasing various autocoids from the endothelium. Activation of endothelial bradykinin receptors increases the formation of the local vasodilator nitric oxide, epoprostenol (also known as prostacyclin and prostaglandin) and platelet activating factor. Therefore, bradykinin, unlike angiotensin II, reduces peripheral vascular resistance through local release of nitric oxide and epoprostenol. Inhibition of ACE may also lead to the accumulation of kinins, thereby potentiating their cardiovascular and metabolic effects. It is likely that both inhibition of angiotensin II production and accumulation of bradykinin contribute to the beneficial effects of ACE inhibitors in human (Catherine V.G. 1997).

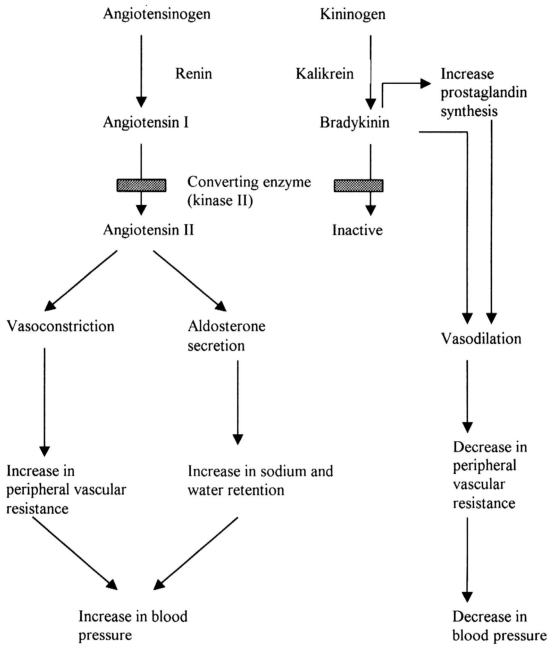

Figure 1.1 Sites of action of ACE inhibitors (adapted from Basic and Clinical Pharmacology, by Bertram G. Katzung, 4th. Edition, p.135, 1989)

1.1.2 Chemical features of ACE inhibitors

Structurally, ACE inhibitors are peptide analogues classified by the chemical group that binds to a zinc ligand at the active site of ACE. These groups may be sulphydryl-containing (e.g. captopril), carboxyl-containing (e.g. enalapril), or phosphorus-containing (e.g. fosinopril). The potency of ACE inhibitors is largely determined by the strength of binding of the zinc ligand and by the number of auxiliary binding sites within the active centers of ACE. Recent molecular biology studies have established the existence of two active sites in the ACE protein, which may be significant with respect to ACE inhibitor binding and substrate selectivity (Linz W. *et al.* 1995). For example, although the carboxylic acid derivatives bind weakly to the principal site of ACE, additional binding sites increase their activity (Allen J.E.1992). Nonetheless, the clinical significance of different binding affinities is unknown. Some chemical-physical properties of the ACE inhibitors are summarized in Table 1.1.

Importance of the sulphydryl group:

It is still controversial as to whether the presence of a sulphydryl group (independent of ACE inhibition) offers a unique advantage. Sulphydryl-containing ACE inhibitor such as captopril may act as scavengers of oxygen-derived free radicals and may therefore be more effective in the treatment of reperfusion injuries (Allen J.E.1992). However, studies have not detected an inhibitory effect of clinically relevant doses of captopril on superoxide radical generation. Captopril and other sulphydryl donors such as acetylcysteine do attenuate the tolerance that occurs with long-term administration of organic nitrates (Unger T. *et al.*1994). The clinical significance of these effects is unclear.

Table 1.1 Chemical-Physical properties of some ACE inhibitors in use

Drug	Zinc Ligand Group	Prodrug	Lipid solubility[a]
Benazepril	Carboxyl	Yes	+
Captopril	Sulphydryl	No	+
Enalapril	Carboxyl	Yes	+ +
Fosinopril	Phosphinyl	Yes	+ + +
Lisinopril	Carboxyl	No	0
Moexipril	Carboxyl	Yes	NA
Perindopril	Carboxyl	Yes	+
Quinapril	Carboxyl	Yes	+ +
Ramipril	Carboxyl	Yes	+
Spirapril	Carboxyl	Yes	+
Trandolapril	Carboxyl	Yes	+ +
Zofenopril	Sulphydryl	Yes	+ + +

[a]+ = slight, + + = moderate, + + + = high, NA = not available

1.2 CLINICAL USES OF ACE INHIBITORS

1.2.1 Hypertension

ACE inhibitors are effective antihypertensive agents for patients with mild to moderate hypertension and are at least as efficacious as ß-blockers, diuretics, and calcium channel blockers (Frampton J.E. *et al.*1995; Leonetti G. *et al.*1995). ACE inhibitors lower systemic vascular resistance without inducing reflex sympathetic activation, which normally opposes the beneficial effects of direct-acting vasodilators. They are not associated with the development of tolerance and do not adversely affect plasma lipid profiles, offering advantages over many other therapies for hypertension.

ACE inhibition appears to be particularly useful for hypertensive patients with diabetes or renal parenchymal disease (Bjorck *et al.* 1986; Opsahl *et al.* 1990). Compared with other antihypertensive agents, ACE inhibitors may offer the advantage of reducing glomerular injury. In patients with diabetes or reduced renal function, ACE inhibitors reduced proteinuria and slows the rate of progression towards end-stage renal failure (Opsahl *et al.* 1990; Taguma *et al.* 1985).

All currently available ACE inhibitors are equally effective in lowering blood pressure when used in equipotent dosages. However, they differ in their duration of action. Current recommendation specify that, to reduce end-organ damage, the trough effect of an antihypertensive agent should be at least 50% of the peak effect (Leonetti *et al.* 1995). Trough:peak ratios higher than 50% are believed to be associated with smoother and more constant blood pressure reduction, which may reduce the incidence of adverse effects that occur in association with peak hypotensive effects.

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The once daily ACE inhibitors differ in their ability to optimally reduce blood pressure over 24 hours (that is, a trough:peak ratio > 50%). Zannad F. (1993) reviewed several trials comparing trough:peak ratios with 24 hour ambulatory blood pressure recordings, in which both smaller and larger dosages of each agent were evaluated. Among the drugs examined, captopril, benazepril, perindopril, ramipril, and quinapril had ratios below 50% with once daily administration, whereas enalapril, lisinopril, and trandolapril had ratios above 50% (Nussberger *et al.*1994).

These results may have clinical relevance concerning the choice of an ACE inhibitor for the treatment of hypertension. Except for captopril, other ACE inhibitors may be administered once daily initially but may require twice-daily administration to achieve optimal control of blood pressure over a 24 hours period.

1.2.2 Congestive heart failure (CHF)

The use of vasodilator agents has become an accepted part of the treatment regimen for symptomatic chronic congestive heart failure (CHF) (Cohn J.N. 1985). The ACE inhibitors have played a central role in achieving this acceptance. Many studies have confirmed the

activation of the renin-angiotensin system in CHF (Levine T.B. *et al.* 1982 ; Goldsmith *et al.* 1983). It was, therefore, reasonable to assume that agents that interfere with the production of angiotensin II might prove beneficial in the treatment of CHF.

Recently, a group of investigators conducted an overview of randomised trials evaluating the effects of ACE inhibitors on mortality and morbidity in patients with symptomatic CHF (Garg R. and Yusuf S. 1995). The overview included all completed, randomised, placebo-control trials of ACE inhibitors that lasted at least eight weeks, regardless of sample size. Altogether, 32 trials conducted in 7105 patients were included. Of these trials, six (n= 697) evaluated captopril, seven (n=3381) evaluated enalapril, six (n= 1227) evaluated ramipril, five (n= 875) evaluated quinapril, and four (n=546) evaluated lisinopril. Benazepril, cilazepril and perindopril were used in one or two trials involving a total of 379 patients. Mortality was the primary endpoint in the two largest trials, CONSENSUS (Cooperative North Scandinavian Enalapril Survival Study) and SOLVD (Studies of Left Ventricular Dysfunction).

Overall, 611 of 3870 patients (15.8%) in the ACE groups died, compared with 709 of 3235 controls (21.9%). The ACE inhibitors were therefore associated with a significant reduction in mortality. The most common cause of mortality was progressive heart failure, followed by sudden or presumed arrythmatic death, myocardial infarction (MI), stroke and pulmonary embolism. The reduction in mortality was mainly due to a decrease in deaths from progressive heart failure.

Data on hospitalisations for CHF were available for 30 of the 32 trials. Among 3810 patients, 22.4% in the active treatment group died or were hospitalised for CHF compared with 32.6%

in the placebo group. The findings of this analysis of the combined trials are consistent with results of SOLVD and CONSENSUS. The benefits observed were consistent among the various ACE inhibitors evaluated. These data provide clear evidence that ACE inhibitors should be used routinely and early in patients with CHF and that sustained, indefinite therapy is warranted.

1.3 THE USE OF CAPTOPRIL FOR THE TREATMENT OF HYPERTENSION AND CONGESTIVE HEART FAILURE

1.3.1 General characteristic of captopril

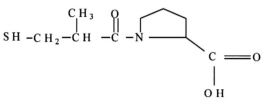

Figure 1.2 Chemical structure of captopril

Captopril (1-[(2S)-3-mercapto-2-methylpropionyl]-L-proline), the first orally active ACE inhibitor, contains a sulphydryl (SH) group that distinguishes it from any other ACE inhibitors. The compound is a white or off-white crystalline powder which may have a characteristic sulphide-like odour. It is freely soluble in water, alcohol, chloroform and in methyl alcohol. The detection limit in plasma is 0.1 ng/ml by gas chromatography-mass spectrometer (Ito *et al.* 1987) and 5 ng/ml by high-performance liquid chromatography (Kawahara *et al.* 1981).

1.3.2 Dosage and administration

Captopril is given by mouth and used alone or in combination (Frewin DB *et al.*1989) for the treatment of mild to moderate hypertension, and in severe hypertension resistant to other treatment. It is also used as an adjunct in the treatment of severe congestive heart failure.

Hypertension

In the treatment of hypertension, an initial oral dose of 12.5 mg is given twice daily, with the dose being increased gradually at intervals of 2 to 4 weeks according to the response (Martindale: The extra pharmacopoeia, pages 346, 30th. Edition, 1993). The usual maintenance dose is 25 to 50 mg twice daily and should not normally exceed 50 mg three times daily even in more severe hypertension, with the addition of second agent, or substitution of an alternative drug (Frewin D.B. *et al.*1989). An initial dose of 6.25 mg twice daily is recommended if captopril is given in addition to a diuretic, to elderly patients, or to those with renal impairment (Martindale: The extra pharmacopoeia, pages 346, 30th. Edition, 1993).

If satisfactory reduction of blood pressure has not been achieved after 1 or 2 weeks, dosage may be increased to 100 or 150mg daily (Brogden *et al.* 1988). Addition of a diuretic should be tried before captopril dosage is further increased. If blood pressure has not been adequately reduced after the dosage of the diuretic is increased to its maximum recommended amount, captopril dosage may be increased further, but must not exceed 450 mg daily (Brogden *et al.* 1988).

Congestive heart failure (CHF)

Initial usage of captopril in the treatment of congestive heart failure will cause severe first dose hypotension and this is especially true for patient on loop diuretics. In addition their

temporary withdrawal may cause rebound temporary oedema (Martindale: The extra pharmacopoeia, pages 346, 30th. Edition, 1993). Thus an initial dose of captopril can be 50 mg daily, given either once daily or in two divided doses. The drug should be given in conjunction with a diuretic, although a much lower initial dosage of 6.25 to 12.5 mg may be required to minimise the magnitude or duration of hypotension in patients who have been vigorously treated with a diuretic and are sodium and/or volume depleted (Brogden *et al.* 1988)

After a dosage of 50 mg three times daily is reached, further dosage increases should be delayed, where possible, for at least 2 weeks to determine if a satisfactory response occurs. Maximum daily dosage should not exceed 450mg (Brogden *et al.* 1988).

1.4 PHARMACOKINETICS OF CAPTOPRIL

1.4.1 Absorption

Absorption is the transfer of a drug from its site of administration to the blood stream. The rate and efficiency of absorption depend on the route of administration. For intravenous delivery, absorption is complete, that is, the total dose of the drug reaches the systemic circulation. Drug delivery by other routes may result in only partial absorption and thus lower bioavailability. For example, the oral route requires that a drug dissolve in the gastrointestinal fluid and then penetrate the epithelial cells of the intestinal mucosa. The presence of food may affect this process.

Captopril is available in oral tablet and is given orally before food. Unlike other ACE inhibitors captopril is active in its orally absorbable form. Captopril is rapidly absorbed from gastrointestinal tract in normal individuals with detectable concentrations being apparent as

early as 15 minutes following administration (Kubo S.H. and Cody R.J. 1985). Approximately 60 to 75% of an oral dose is absorbed (Duchin *et al.* 1982b ; Kripalani *et al.* 1980). In two way cross-over study in which 5 healthy subjects received 10 mg ^{14}C-captopril as an oral solution and intravenously on another occasion, absolute absorption of the radioactive dose average 71% (unchanged drug plus biotransformation products), and the bioavailability of unchanged captopril was 62% based on ratios (oral:intravenous) from urinary excretion data (Duchin *et al.*1982). As absorbed captopril is excreted almost exclusively by the kidney, urinary excretion of the total drug is a good indicator of absorption of the drug.

In another two studies involving 100 mg single oral dose of radiolabelled captopril in fasting subjects, mean maximal blood concentrations (C_{max}) for unchanged captopril were about 0.8 to 0.9 mg/L and that for total captopril (captopril plus metabolites) was about 1.6 to 1.9 mg/L with excretion of total radioactivity averaging 65 % (Kripalani *et al.* 1980) and 76% (Singhvi *et al.* 1982). The values were similar to those found in the oral-intravenous crossover study with 10 mg doses (Duchin et *et al.*1982).The results of these studies and others (Duchin *et al.* 1982; Mantayla *et al.*1984; Onoyama *et al.* 1981) showed that C_{max} and AUC (area under the plasma concentration-time curve) values generally appear to be directly related to dose over the range from 10 to 100 mg captopril.

After oral administration of 100 mg captopril in the fasted state, the maximum blood concentration (C_{max}) of unchanged captopril in blood occurred at 0.6 to 0.9 hours post dose (Kripalani *et al.*1980; Massana E. *et al.* 1997 ;Franklin M.E. *et al.* 1998). An intermediate dose of 50 mg also showed that the time to reach C_{max} (T_{max}) was 0.7 hours (Onoyama *et al.* 1981; Jankowski A. *et al.* 1995; Ahmed *et al.* 1996). A pharmacokinetic study on 12 healthy

subjects given 25 mg captopril orally produced C_{max} and T_{max} values of 162 ± 72.1 ng/ml and 0.63 ± 0.23 hours, respectively (Jankowski A. *et al.* 1995). A pharmacokinetic study for 10 mg captopril by Duchin *et al.* (1982) also produced the maximum blood concentration at 0.7 ± 0.05 hours.

1.4.1.1 Effect of food

Intake of food at the time of captopril administration can slow down its absorption and thus change the pharmacokinetic profile of captopril. Singhvi and co-workers, (1982) and Mantyla and co-workers, (1984) demonstrated that ingestion of food can reduce the bioavailability of captopril by about 35 to 55%. It has been suggested that the constituents of food may form disulphides with captopril, which may then not be absorbed, or that the elevation in gastric pH may promote ionisation of captopril, thus lowering its absorption. In another one study, William *et al.*, 1982 reported that food can be consumed 1 hour after captopril administration without affecting its bioavailability.

Despite the lower circulating concentrations of unchanged and total captopril when captopril was taken with food, its antihypertensive effects have not been compromised (Muller *et al.* 1985; Ohman *et al.* 1985; Salvetti *et al.* 1985). Ohman reported that peak plasma concentration and AUC of total and non protein-bound captopril were significantly reduced by concomitant food intake. Muller and co-worker, (1985) noted that both C_{max} and AUC from 0 to 120 hours, were reduced when food was ingested at the time of captopril administration.

1.4.1.2 Effect of age

Creasey *et al.* (1986) reported pharmacokinetic parameters for single 100 mg oral doses of captopril in healthy elderly (>65 years) male subjects with normal renal function. These values were similar to those found after the same dose was given to young healthy subjects in the fasted state (Kripalani *et al.*1980; Singhvi *et al.*1982) using the same assay method and study design. Thus, age is not a reason for dosage adjustment.

1.4.1.3 Gender effect

In one study, 100 mg captopril were given orally to 12 males and 12 females under fasting condition (Massana E. *et al.* 1997). Mean plasma concentrations showed no statistical differences between males and females throughout the time course assessed. In addition, no statistically significant gender differences were found in any other pharmacokinetic parameters. The pharmacokinetic parameters for captopril in both males and females are shown in the Table 1.2:

Table 1.2: Captopril pharmacokinetic parameters in males and females after a 100 mg oral dose (Massana E. *et al.* 1997)

	C_{max} (ng/ml)	T_{max} (h)	AUC (ng.h /ml)	$t_{1/2}$ (h)
Males	586.71±235.83	0.90±0.47	718±213.73	1.13±1.45
Females	553.66±229.89	0.92±0.43	728±181.03	0.88±0.39

1.4.2 Distribution

Drug distribution is the process by which a drug reversibly leaves the blood stream and enters the interstitium (extracellular fluid) and or the cells of the tissues. The delivery of a drug from the plasma to the interstitium depends on blood flow, capillary permeability, the degree of binding of the drug to plasma and tissue protein, and relative hydrophobicity of the drug.

Whole-body autoradiography in rats showed that captopril and its metabolites are rapidly distributed to most tissues with the exception of the central nervous system (Heald & Ita,1977). In various animal species captopril has been shown to cross the placenta (Davidson *et al.* 1981; Pipkin *et al.* 1982). It is uncertain from individual case reports whether this occurs in humans (Boutroy *et al.* 1984; Fiocchi *et al.* 1984).

Captopril is about 30% covalently, but reversibly, bound to protein in human blood (Maeda *et al.* 1979; McKinstry *et al.* 1978). In healthy subjects the mean volume of distribution at steady state was 0.7 to 0.75 L/kg (Duchin *et al.* 1982; Singhvi *et al.* 1982a; Devlin and Feiss, 1981). Duchin *et al.* (1982) compared the pharmacokinetics of captopril after oral and intravenous administration. The data fitted a 3-compartment model, indicating 2 peripheral compartments. The mean volume of the central compartment was 0.22 L/kg. However, the mean volume of distribution during the terminal phase was 2.05 L/kg, which was greater than the volume of total body water and hence indicated extensive partitioning of the drug into tissues.

In another two studies involving hypertensive patients, the volume of distribution reported were 0.58 L/kg (Giudicelli *et al.* 1984) and 0.64 L/kg (Richer *et al.* 1984). The volume of distribution is a hypothetical volume of fluid into which the drug is disseminated. Although the volume of distribution has no physiological or physical basis, it is sometimes useful to compare the distribution of a drug with the volumes of the water compartments in the body. Volume of distribution (V_d), often expressed in units of litres or litres/ kilogram, may be estimated from Equation 1:

$$V_d = \frac{\text{Amount of drug in body}}{C} \quad \ldots\ldots\ldots \text{(Eqn. 1)}$$

Where C = plasma concentration of drug

1.4.3 Metabolism and excretion

In its passage through the body and particularly through the liver, the drug is acted upon by enzymes which metabolise it and convert the original active drug to various metabolites which are, in general, inactive, although there are some drugs whose metabolites are thought to have activity similar to that of the original "parent" drug. A particular problem with orally administered drugs is the so called first pass effect in which the absorption of the drug into the blood stream via the portal vein to the liver and thence to the blood stream may result in extensive metabolism.

Once captopril is absorbed, it is metabolised to several forms, including a disulphides dimer of captopril, a captopril-cystein disulphide, and other mixed disulphides with endogenous thiol compounds (cystein, glutathione)(Komai *et al.* 1981; Wong and Dreyfuss, 1978; Stanislaw Sypniewski & Edward Bald, 1996). It is likely that captopril and its pool of metabolites undergo several reversible interconversions.

The key to the disposition and biotransformation of captopril is its free sulphydryl group which binds to endogenous thiol-containing compounds *in vivo.* Wong *et al.* (1981) reported that captopril covalently reacts with human and rat plasma proteins when incubated *in vitro.* This type of binding has been confirmed by ultrafiltration, acid precipitation techniques (Wong *et al.* 1981) and equilibrium dialysis (Park *et al.* 1982). When radiolabelled captopril was added to human plasma *in vitro*, the binding of captopril, principally to albumin, increase with time, reaching 28% after 1 hour (Park *et al.* 1982). Captopril however does not bind to macromolecules in blood cells, regardless of the length of incubation. Unchanged captopril concentrations in a suspension of washed blood cells remained relatively stable over 2 hours incubation period, indicating little or no biotransformation in the blood (Wong *et al.* 1981).

It is well known that thiol-containing compounds will undergo oxidation process to disulphides. Cystein and glutathione, which are maintained intracellularly predominantly in the reduced form, are the most abundant cellular free thiol-containing compounds. On the other hand, the disulphides tend to predominate extracellularly and are primarily associated with albumin and other plasma proteins. The disulphide linkages between captopril and plasma proteins can be cleaved by the addition of glutathione, cystein, and thiothreitol, a reducing sulphydryl compound. The addition of glutathione or cystein releases protein-bound captopril by displacement, and increases the concentration of glutathione- and cystein- mixed disulphides of captopril (Wong *et al.* 1981). Furthermore, the glutathione mixed disulphides may release unchanged captopril via hepatic thioltransferases or may be hydrolised by γ-glutamyltranspeptidase and dipeptidase in the kidney to give captopril-cystein mixed disulphide, a major metabolite of captopril (Park *et al.* 1982).

In healthy subjects, Kripalani *et al.* (1980) reported that about 38% of an oral dose of captopril was excreted in urine as the unchanged form and 1.5% was present as captopril disulphide during the 24 hours after administration of ^{14}C-captopril. Within the first 2 hours of drug administration captopril metabolites comprised about 27% of the radioactivity recovered in the urine, rising to nearly 75% in the 12 to 24 hours interval. The time course of captopril and biotransformation products in human blood after oral administration was similar to that found after intravenous administration.

Drummer *et al.* (1982) reported that the excretion of S-methyl captopril accounted for about 1% after 6 hours in the urine of hypertensive patients with normal renal function receiving long term captopril, but not after a single dose. However, Creasey *et al.* (1986) found this

metabolite in plasma after a single oral dose of captopril in healthy elderly subjects (65 to 76 years of age).

In another study, Sloand and Izzo (1987) reported captopril treatment (75 or 150 mg/day) was effective in reducing urine cystein excretion in 2 patients with cystinuria. It is presumed that the formation of captopril-cystein mixed disulphide, which is 200 times more soluble than cystein, contributed to the decrease in cystein excretion.

1.4.4 Clearance

Clearance (CL) is a descriptive term used to evaluate efficiency of drug removal from the body. It is defined as the ratio of the rate of elimination by all routes to the concentration of drug in a biological fluid. It is often expressed in units of litres/hour /kilogram, and may be estimated from equation 2:

$$CL = \frac{\text{Rate of elimination}}{C} \quad \text{..... (Equation. 2)}$$

Where C = plasma concentration of drug

The value reported for total body clearance for captopril were varied among the studies. The clearance values for captopril from some studies are tabulated in the Table 1.3:

Table 1.3 Literature values of some body clearance for captopril

	CL /F(l/hr.kg)
Duchin *et al.* (1981)	0.76 ± 0.08
Richer *et al.* (1984)	0.69 ± 0.08
Creasey *et al.* (1986)	0.19 ± 0.02
Jankowski *et al.* (1994)	72.92 ± 18.53*b
Jankowski *et al.* (1994)	97.77 ± 22.34*b
Massana *et al.* (1997)	2.36 ± 0.67

*b = unit in l/hr, assume average weight of 70 kg than the CL/F would be 1.04 to 1.40 l/hr.kg

Three early literatures by Duchin *et al.* (1981), Richer *et al.* (1984), and Creasey *et al.* (1984) have a lower CL as compared to others. This might be due to the assay sensitivity at that time ~~might not be~~ as good as recent study.

1.4.5 Elimination half-life

Half-life ($t_{1/2}$) is the time required to change the amount of drug in the body by one half during elimination. In the simplest case, the body may be considered as a single compartment of a size equal to the volume of distribution (V_d). While the organs of elimination can only clear drugs from the blood or plasma which is in direct contact with the organ, this blood or plasma is in equilibrium with the total volume of distribution. Thus, the time course of drug in the body will depend on both the volume of distribution and the clearance as given by equation 3:

$$t_{1/2} = \frac{0.693 \times V_d}{CL} \quad \text{(Eqn. 3)}$$

The half-life for captopril is reported to be about 1 to 2 hours. The half-life values which were reported in some literatures are summarized in Table 1.4:

Table 1.4 Literature values of some half-life for captopril.

	$t_{1/2}$ (h)
Duchin *et al.* (1981)	1.93 ± 0.20
Creasey *et al.* (1986)	1.40 ± 0.10
Jankowski *et al.* (1994)	1.11 ± 0.34
Jankowski *et al.* (1994)	1.03 ± 0.19
Massana *et al.* (1997)	1.01 ± 0.92
Franklin ME. *et al.*(1998)	1.58 ± 0.41

1.5 PHARMACOKINETICS OF CAPTOPRIL IN PATIENTS WITH HYPERTENSION AND CONGESTIVE HEART FAILURE

1.5.1 Hypertension

The pharmacokinetics of captopril in patients with hypertension and congestive heart failure (CHF) have been less extensively evaluated. One would not expect to see major differences in the pharmacokinetics of captopril in patients with hypertension as compared to healthy individuals, except in those patients with secondary renal dysfunction as they are 38% excreted unchanged (Kripalani *et al.* 1980). By contrast there are many reasons for the expected marked alterations in the pharmacokinetics of captopril in patients with CHF, as a consequence of the many pathophysiological abnormalities associated with systemic vasoconstriction and total body fluid overload.

In one single dose study, Richer *et al.* (1984) studied 10 patients with essential hypertension who were given captopril 1 mg/kg orally in the fasting state. The kinetics of captopril plasma concentrations, plasma rennin activity and blood pressure were followed for 24 hours. Concentrations of unchanged captopril were measurable as early as 20 minutes after

administration.The values for C_{max}, T_{max}, volume of distribution and elimination half-life are very similar to that observed in healthy individuals, as reported Kripalani *et al.* (1980) and Duchin *et al.* (1982). Six hours post dose, captopril could only be detected in plasma in 2 patients. After 24 hours it could not be detected in plasma in any of the patients studied (Richer *et al.* 1984).

In multiple dosing studies, the effect of long term administration on the pharmacokinetic properties of captopril in hypertensive patients have been inconsistent. McKinstry and co-workers (1980) studied the effects of 100 mg captopril administered every 8 hours for 10 days in hypertensive patients with normal renal function. There is no difference in C_{max}, T_{max}, or AUC for unchanged captopril and total radioactivity between day1 and day 10. In contrast, Jarrot and co-workers (1982) evaluated pharmacokinetic properties of a 100mg oral dose of captopril in patients with hypertension who had been taking captopril for at least 6 months (multiple-dose group). The patients in the multiple-dose group had a higher C_{max} and earlier T_{max} compared with the patients who had never taken captopril before. The AUC was significantly higher and elimination half-life was unchanged in the multiple-dose group compared with the patients who had never taken captopril before. These differences suggested that the bioavailability of captopril increased with multiple-dose administration. The mechanism of this effect, while not clear, is probably related to reversible interconversions between free captopril and its pool of metabolites.

1.5.2 Congestive Heart Failure (CHF)

Patients with CHF frequently have mucosal wall oedema of the gastrointestinal tract and marked reductions in splachnic blood flow, which contribute to the limitation of gastrointestinal absorption of all orally administered drugs (Covit *et al.* 1985). Renal blood

flow is also frequently reduced, with concomitant reductions in renal function and glomerular filtration rate (Covit *et al.* 1985), which contribute to a prolonged half-life for drugs that are primarily excreted by the kidneys. Since there is a wide range of severity of these abnormalities, it is difficult to obtain accurate information on the pharmacokinetics of captopril in CHF.

Single dose pharmacokinetics have been studied in 12 patients with chronic CHF who were each given a single 25 mg oral dose of captopril in the fasting state (Cody *et al.* 1982). The C_{max}, T_{max}, AUC and elimination half-life are very similar to those observed in normal subjects and in patients with hypertension, except for a slightly delayed T_{max}, suggesting that, despite the number of potential conflicting influences, the pharmacokinetics of captopril are relatively preserved in CHF. Half-life reported in this study was 1.4 hours, while clearance and volume of distribution were not reported in this study.

In another study involving oral administration of captopril to 20 patients with CHF, haemodynamic responses and plasma concentrations of unchanged captopril were assessed after a single 25 mg dose (Shaw *et al.* 1985). Absorption was rapid in many of these patients, with mean peak plasma concentrations of unchanged captopril occurring at 45 minutes after the dose. However, several patients had slow absorption with peak concentrations at 90 to 240 minutes. These investigators reported a smaller second peak in the plasma concentration curve, which has not been apparent in other studies. The mean elimination half-life in 6 patients was reported to be 7 hours.

1.6 ADVERSE EFFECTS OF CAPTOPRIL

Captopril is generally well tolerated at doses below 150 mg daily. Clinical experience with captopril has involved many thousands of patients studied in formalised trials. The initial use of captopril was characterised by a relatively high incidence of side effects because the drug was frequently used in patients with severe hypertension and concomitant renal function impairment or collagen vascular disease, particularly when high dosages of captopril were given. More recent experience with lower dosages of captopril in many thousands of patients with mild to moderate hypertension, many of whom had normal renal function, clearly demonstrated a lower incidence of side effects (Chalmers *et al.* 1987; Edwards *et al.* 1987; Jenkins *et al.* 1985a).

Adverse effects tend to be dose-related and more frequent in patients with impaired renal function. In postmarketing surveillance studies the incidence of the most commonly reported side effects varied according to dosage and pre-existing renal function (Groel *et al.* 1983 ; Jenkins *et al.* 1985a), generally being higher with daily dosages exceeding 150 mg and in patients with pre-existing renal disease. The commonest adverse effects are skin rashes which may be accompanied by pruritus during the first few days or after an increase in dosage. It may be accompanied by fever, arthralgia, and eosinophilia. Also described are taste disturbance. Loss of taste was the most frequently reported disturbance noted by Edwards *et al.* (1987). A non-productive cough, frequently described as an irritating sensation in the throat, has been reported with captopril and other ACE inhibitors. The cough which usually occurs after several weeks of treatment, generally resolves within several days of discontinuation of captopril.

Proteinuria has been observed with low frequency (less than 2% of the patients on high dosages) in patients with and without underlying renovascular disease. Risk factors for neutropenia and proteinura are the presence of a collagen vascular disease, dosage of captopril of more than 150 mg/day, and decreased renal function. Proteinuria usually reverses upon with withdrawal or discontinuation of the drug (Jenkins *et al.* 1985a).

Hypotension usually occurs early in the course of treatment, often after the first dose (Hodsman *et al.* 1983), particularly in patients with congestive heart failure and in sodium-depleted patients (for example, those who have received previous diuretic therapy). This can be minimised by starting with a low dose of captopril and giving the initial dose at night. Alternatively, patients may take a diuretic "holiday".

Captopril may also cause lymphadenopathy, anaemia in children, a positive antinuclear antibody test and false-positive test for urine acetone. Cholestatic jaundice, headache, and insomnia have been reported but are rare (Rahmat *et al.* 1985). Captopril should not be given during pregnancy because there have been instances of foetal reabsorption, growth retardation, and hypotension at birth.

1.7 AIM OF THE STUDY

The tolerability of captopril has now been studied in many thousands of patients who have been involved in formalised trials and the early impression of poor tolerability can no longer be justified. Thus, captopril with its generally good tolerability and proven long term efficacy warrants consideration as a 'first line' therapy in patients with mild or moderate essential hypertension and as a drug of choice in patients with chronic congestive heart failure when digitalis is poorly tolerated or ineffective.

The pharmacokinetic properties of captopril are very clear with a lot of published data. The pharmacokinetics of captopril has been well studied in healthy subjects, in patients with hypertension and in patients with congestive heart failure. The effects of renal impairment and age on its disposition are also well documented.

The aim of this study is to develop a rapid and sensitive method for the analysis of captopril and to use the method for four sets of bioequivalence studies of generic captopril. The bioequivalence studies will be conducted for four generic products with one brand leader as a control. A total of more than 60 volunteers will be involved in the bioequivalence studies. From the results of the bioequivalence studies, a population analysis of the pharmacokinetics (standard two stage method) of captopril will be carried out.

1.8 POSSIBLE OUTCOMES OF THE STUDY

A method for the analysis of captopril will be developed and this will be used to analyse captopril for the bioequivalence studies. The information obtained from the bioequivalence studies will be released to the pharmaceutical company. Information which would attest to the quality of the newly-formulated drug preparation can be used to inform the relevant authorities eg. the Drug Control Authority (DCA), doctors who are likely to prescribe the drugs, as well as pharmacists, of the safe substitution of the generic for the brand-name product or vice versa, should the need arise. On the other hand, if the newly formulated drug preparation is found not to be equivalent, the release of the drug into the market will be withheld until the formulation is improved.

The population analysis of the pharmacokinetic of captopril will determine whether the values obtained from the bioequivalence studies falls within the normal range and whether the values are within the values obtained from published data.