VALIDATION OF ASSAY METHODS FOR CHLORPROGUANIL AND CHLORCYCLOGUANIL AND ITS APPLICATION TO PHARMACOKINETICS STUDIES

by

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Thesis submitted in fulfillment of the
requirements for the degree
of Master of Science

April 1996

ACKNOWLEDGEMENTS

1. I would like to acknowledge the financial support provided by the graduate assistantship scheme of the Centre for Drug Research, Universiti Sains Malaysia.

2. I am grateful to my supervisors, Professor (Dr) V. Navaratnam and Dr. Sharif Mahsufi Mansor for their valued guidance, support and helpful scientific discussion throughout the research.

3. I wish to thank Professor W.H. Wernsdorfer for his continued interest and support throughout the research project.

4. I would also like to thank all the staff and my colleagues of the Centre for Drug Research, Universiti Sains Malaysia for their help , support and friendship.

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ABSTRACT

Two assay methods have been developed and validated for the analysis of chlorproguanil and chlorcycloguanil. The gas chromatographic procedure with electron-capture detector (GC-ECD) was developed for the analysis of chlorproguanil while a reverse phase liquid chromatographic procedure with ultra-violet detector at 235nm (HPLC-UV) was developed to assay for chlorcycloguanil alone.

The gas chromatographic procedure developed, using proguanil as the internal standard, gave good resolution for proguanil and chlorproguanil. A linear response was obtained over the range 1.0 - 50.0ng. The average recovery for chlorproguanil was 80.8 ± 5.4%. Extraction recoveries were linear for chlorproguanil within the range 2.0-40ng/ml (coefficient of correlation = 0.996). The within-day precision for chlorproguanil gave coefficient of variation between 4.9% - 7.9% and day-to-day precision was between 0.72 - 7.3%. The assay sensitivity was 0.1 ng/ml for chlorproguanil.

In the HPLC procedure cycloguanil was used as the internal standard. Detector linearity for chlorcycloguanil was obtained over the range 1.0 - 80 ng. The average recovery of chlocycloguanil was found to be 99.4 ± 5.6%. The

within-day coefficient of variation was between 1.8% - 6.8% and day-to-day was 4.5 - 9.2%. The limit of assay sensitivity by HPLC-UV was 0.5ng/ml.

The pharmacokinetic study on healthy Malaysian volunteers was carried out. Chlorproguanil is well tolerated and safe in all subjects receiving 20 mg of Lapudrine tablet administered as a single oral dose. In our study one subject appears to absorb chlorproguanil to a limited extent. The mean C_{max} for chlorproguanil was 25 $\pm$ 4 ng/ml and the mean t_{max} was 3.0 $\pm$ 1.2 hour. Chlorproguanil has a mean $t_{1/2}$ of 37.1 $\pm$ 19.5 hour and a mean plasma CL of 0.93 $\pm$ 0.26 L/hr/kg. The mean Vd was 51.1 $\pm$ 38.1 L/kg while the mean $AUC_{0-\infty}$ 365 $\pm$ 101 ng hr/ml was observed. Chlorcycloguanil was measurable in only two subjects and the mean $AUC_{0-\infty}$ in these subjects was 147.6 $\pm$ 11.0 ng hr/ml. The mean C_{max} and t_{max} for all subjects were 6.7 $\pm$ 5.0 ng/ml and 5.6 $\pm$ 3.0 hour respectively. Our study indicates that there are slight pharmacokinetic differences of chlorproguanil in Malaysian volunteers as compared to those derived in the Caucasian subjects. Factors such as dose , size , weight of the subjects and genetics may contribute to the differences in the pharmacokinetic parameters of chlorproguanil in healthy volunteers.

PENGESAHAN KAEDAH BAGI PENGESEIAN CHLORPROGUANIL DAN CHLORCYCLOGUANIL DAN APLIKASINYA KEPADA KAJIAN FARMAKOKINETIK

ABSTRAK

Dua kaedah pengeseian telah dikembangkan dan disahkan bagi analisis klorproguanil dan klorsikloguanil. Kaedah gas kromatografi penangkap elektron (KG-ECD) telah dikembangkan bagi pengeseian chlorproguanil sementara kaedah kromatografi cecair keupayaan tinggi fasa terbalik dengan pengesan ultra lembayung pada 235nm (KCKT-UV) telah dikembangkan bagi pengeseian klorsikloguanil sahaja.

Didalam kaedah gas kromatografi yang dikembangkan, proguanil digunakan sebagai piawai dalaman. Proguanil dan klorproguanil didapati menunjukkan resolusi yang baik didalam pemisahan yang dijalankan. Keluk tentukuran bagi pengesan adalah bergaris linear bagi pengesanan dalam julat 1.0-50.0ng. Purata peratus pengembalian bagi klorproguanil adalah 80.8 $\pm$ 5.4%. Keluk tentukuran piawai bagi klorproguanil adalah linear bagi julat kepekatan 2.0-40ng/ml (pekali koefisien = 0.996). Peratus pekali ubahan bagi kepresisan dalam sehari bagi klorproguanil adalah diantara 4.9% - 7.9% and kepresisan dari hari ke hari adalah diantara 0.72 - 7.3%. Had pengesanan bagi

klorproguanil melalui KG-ECD adalah 0.1 ng/ml.

Dalam kaedah kromatografi cecair keupayaan tinggi yang dikembangkan sikloguanil telah digunakan sebagai piawai dalaman . Kelinearan pengesanan telah didapati bagi julat 1.0 - 80 ng. Purata peratus pengembalian klorsikloguanil adalah 99.4 $\pm$ 5.6%. Peratus pekali ubahan bagi kepresisan dalam sehari adalah diantara 1.8% - 6.8% dan bagi dari hari ke hari adalah 4.5 - 9.2%. Had pengesanan klorsikloguanil melaui kaedah KCKT-UV adalah 0.5ng/ml.

Kajian farmakokinetik keatas sukarelawan sihat Malaysia telah dijalankan. Toleransi klorproguanil adalah baik dan selamat bagi kesemua subjek yang menerima tablet 20 mg Lapudrine yang diberikan secara oral. Dalam kajian ini satu subjek didapati tidak menyerap drug ini dengan baik. Purata C_{max} bagi klorproguanil yang diperolehi ialah 25 $\pm$ 4 ng/ml dan purata t_{max} adalah 3.0 $\pm$ 1.2 jam. Purata $t_{1/2}$ pula ialah 37.1 $\pm$ 19.5 jam dan purata CL bagi plasma adalah 0.93 $\pm$ 0.26 L/jam/kg. Purata Vd adalah 51.1 $\pm$ 38.1 L/kg sementara purata bagi $AUC_{0-\alpha}$ adalah 365 + 101 ngjam /ml. Klorsikloguanil hanya dapat ditentukan pada dua orang subjek sahaja dan purata $AUC_{0-\alpha}$ bagi subjek-subjek ini adalah 147.6 $\pm$ 11.0 ng jam/ml. Purata bagi C_{max} dan t_{max} kesemua subjek adalah 6.7 $\pm$ 5.0 ng/ml dan 5.6 $\pm$ 3.0 jam. Kajian ini telah menunjukkan terdapat sedikit perbezaan farmakokinetik bagi klorproguanil diantara sukarelawan sihat Malaysia dengan subjek dari golongan Kaukasian.

Kaukasian. Faktor-faktor seperti dos, saiz , berat subjek dan genetik seseorang boleh mempengaruhi parameter farmakokinetik klorproguanil bagi sukarelawan sihat ini.

DEDICATION
Dearest mother
Supporting husband
and
loving Aazhimah & Aazeez

1.0 Introduction

1.1 Extent of the disease

Malaria is one of the oldest disease mentioned in the early writings of Egypt, India and China. The clinical symptoms were fully described decades ago by Hippocrates, but until today the outcome of malaria infection is still unpredictable.

Malaria is caused by *P. falciparum* the most deadly species of parasite. Eventhough malaria is not as striking as AIDS, it is still affecting hundreds of millions of people. Between 1-2 millions will die from it in the next decade (Time,1993).

For many years, malaria control by chemotherapy give promising result. The powdered roots of Ch'ang Shen (*Dichron Lebrifuges*) and *Qing hao* (*Artemisinia annua*) was used in China as treatment for at least 2000 years. Only in the seventeenth Century the new remedy was discovered ; the isolation of the alkaloids from the *Cinchona* bark which soon established various salts of quinine, the first potent remedy against malaria. The inability to supply quinine to meet the great demand during the first and second world wars brought about a new development of other synthetic antimalarial drugs. Scientists started searching for new

drugs and began to understand the pathology of malaria. They produced a library of invaluable antimalarial compounds such as 8-aminoquinoline series, 4-aminoquinoline, the dihydrofolate reductase inhibitors and etc. Chloroquine, derived from quinine, is a miracle drug developed during the war which was a relatively inexpensive prophylactic and treatment drug that saved many lives.

The vector control via the introduction of effective insecticides such as dichlorodiphenyltrichloroethane (DDT) also helped to combat the disease and these were then endorsed by WHO in the concept of malaria eradication programme. Vector control which was given an integrated plan in 1956 together with the successful use of the drug, chemoprophylaxis almost controlled malaria in 1950s.

However, a new and menacing event in the history of malaria occurred in 1960s. The discovery that P. falciparum appeared to build up resistance to chloroquine and the increasing number of reports in the resistance of human plasmodia to other prophylaxis drugs such as proguanil and pyrimethamine had caused some disappointment in chemotherapy (Bruce-Chwatt, 1986) . Also, DDT was ineffective in many areas , owing to differing habits of Anopheles which has developed resistance to the insecticide (Warhurst, 1987). This causes the loss of effectiveness in vector control.

The threat posed by resurgent malaria in many parts of the world becomes critical as such the WHO has adopted a global strategy. Eradication, the dogma of the 1960s, is no longer considered realistic. Since malaria occurs under differing conditions, WHO is seeking to have malaria control integrated into government planning in health care, sanitation and development projects, which have sometimes served to help spread the disease (Time, 1993).

Drugs, however still play a key role in the control of malaria particularly in preventing death and reducing morbidity. This situation has stimulated research into a better and more effective usage of the currently available drugs, the development of new antimalarial agents and the introduction of drug combinations.

1.1.1 Malaria life cycles

Most antimalarial drugs target on the particular stages of development of the malaria parasites. Knowledge of its life-cycle is important for the proper deployment of these antimalarial drugs. The true malaria parasites belong to the genus Plasmodium(P) with 4 pathogenic species, P. falciparum, P. malariae, P. ovale and P. vivax. The human plasmodia exhibit two types of schizogony (exoerythrocytic without pigment and erythrocytic with pigment) in the human host and a sexual stage terminating

in sporogony in Anopheles mosquito.

During the blood meal, the sporozoites which reside in the salivary gland of the mosquito is injected into the blood stream and enters the liver parenchymous cells and undergoes one cycle of asexual division to form multi-nucleate shizonts. The pre-erythrocytic tissue schizogony state is completed when the tissue schizonts undergo endoplasmic fission, producing thousands of uninucleate merozoites and causes rupture to the liver cells. In this form, the parasite invades the erythrocytes, grows and multiplies cyclically from trophozoites to mature blood schizonts. Malaria pigment (haemozoin) is produced within the parasite during this stage by the breakdown of the erythrocytes haemoglobin causing a fever which is the symptom of the disease. Some intra-erythocytic forms develop into male or female of sexual parasites (gametocytes), which will unite to form zygote on entering the stomach of the mosquito. Eventually, after the gradual stages of ookinete and oocyts, large numbers of sporozoites are produced and stored in the salivary glands of the Anopheles, which will be transmitted in the next blood meal. In most strains of P. vivax and apparently P. ovale the sporozoites which enter the hepatic cells may delay their development and remain as dormant hypnozoites. After responding to an unknown stimulus or to a preprogrammed genetic message they begin to give rise to merozoites which escape from the liver and infect

erythrocytes whish caused relapse attacks (Krotoski 1985).

For P.malariae and P. falciparum, some recent evidence show that schizogony in these species and the 'recrudescence' may originate from erythrocyctic forms remaining in the body for a considerable time. Relapse of vivax, and ovale malaria may occur at intervals for 3 or 4 years while the 'recrudescence' in P. falciparum infection seldom occur more than 1 year after the primary attack but P. malariae may persist in the blood for 20 - 50 years.

1.1.2 Site of action of antimalarial drugs

A biological classification of antimalarial given here is largely based on the outline by Warhurst (1987).

1. Tissue schizontocides inhibit the growth of the parasite in the liver cell. Drugs used are the 'causal prophylactics' and this include the dihydrofolate reductase inhibitors, proguanil and pyrimethamine which were used alone or in combination and sulphonamides.

2. Hypnozonitocides (Peters, 1983) kill the dormant liver stages (hypnozoites) and used as 'antirelapse' drugs in P. vivax and P. ovale. Primaquine and other 8-aminoquinoline are the only examples that have been found effective in man.

3. Blood schizontocides act rapidly only on the erythrocytic stage and are generally attached in therapy. The drugs acting at this stage with fast action are the cinchona alkaloids (quinine, quinidine), 4-aminoquinolines (chloroquine), the sesquiterpene lactones (eg. artemisinin, artemether, arteether and sodium artesunate), the novel 4-quinolinemethanols (mefloquine) and the phenanthrenemethanols (halofantrine). These drugs have a marked action while dihydrofolate reductase inhibitors (pyrimethamine, proguanil, chlorproguanil), sulphonamides, sulphones, antibiotics and to a small degree primaquine also have a relatively slow blood-schizontocidal effects which also act on other stages.

4. Gametocides will destroy the sexual stages of the parasites in the blood including the mature gametocytes of P. falciparum. The 8-aminoquinoline drugs, especially primaquine are used for this purpose.

5. Sporontocides refer to the inhibitory action of the drug or its metabolite in the blood meals on the subsequent development of the oocycts and sporozoites in the mosquito. Drug with this action include the dihydrofolate reductase inhibitors (pyrimethamine and proguanil). 'Sporontocide' when fed directly to mosquito will inhibit oocycts and sporozoite growth (pyrimethamine and cycloguanil).

1.1.3 Drug resistance in malaria

Drug resistance in malaria has been defined as the ability of a parasite strain to survive and/or to multiply despite the administration and absorption of a drug given in doses equal to a higher than those usually recommended but within the limits of tolerance of the subject (Bruce-Chwatt, 1986).

Resistance by Plasmodium to individual antimalarial compounds has been reported in many parts of the world and often involves cross-resistance to the other compounds. Drugs that Plasmodium was found to be resistant in many parts of the world are the DHFR inhibitors, 4-aminoquinoline (chloroquine, amodiaquine) , quinine, primaquine, mefloquine, sulphonamides and sulphones (Bruce-Chwatt, 1986).

Resistance of P. falciparum. to chloroquine is believed to have developed in 1957 on the Thai-Cambodian border which spreads in all epidemiologically-feasible directions eastwards across the Indo China peninsula and westwards to Thailand within three years (WHO, 1987). Resistance to amodiaquine, a 4-hydroxyaniline-substitute quinoline, largely follows the distribution of chloroquine resistance (Wernsdorfer, 1991). Under continuing chloroquine pressure in the core area, the parasite also showed a diminishing

susceptibility to amodiaquine, quinine, sulphadoxine-pyrimethamine and mefloquine when these drugs were used during the ensuing twenty years (WHO, 1987). The cross resistance between proguanil, chlorproguanil and pyrimethamine has also been reported from different areas, although the pattern of cross-resistance is not uniform (WHO,1987).

Drug resistance are the results of interactions of the biological and epidemiological factors (Wernsdorfer, 1991). The degree of resistance to schizontocidal drugs has been classified into three categories with recommended symbols R1, R11 and R111. R1 pattern is defined as a recrudescence of parasites and symptoms developed some days after chloroquine treatment; R11 resistance is defined as a slight reduction but not elimination of parasitaemia with treatment and R111 is defined as the response to treatment (Bruce-Chwatt,1986).

Malaria parasites have developed mechanisms of resistance against virtually every drug that has been used against them (Peters, 1982,1987; Bjorkman & Phillips-Howard ,1990). Therefore, steps must be taken to limit the further spread of resistance not only to the existing antimalarials but also to drugs under development.

1.1.4 Status of malaria in Malaysia

Malaria is still an endemic disease of public health importance in Malaysia. The areas where outbreaks of malaria occur are mainly confined to the central areas of the country which is hilly and undeveloped . The incidence of malaria declined from 61,593 cases with 223 deaths in 1971, to 44,488 cases with 53 deaths in 1980 (Singh,1985; Chooi, 1985). Malaria incidence from 1982 to 1987 (up to July) was 24,228 cases with 43 deaths, showing a decline on the death cases (Uma & Chee ,1988) ; by Jan-Sept 1993 (recently summarized malaria cases showed in index) the incidence was 28,881 cases with 12 deaths. 25.8% of the total cases were from peninsular Malaysia, 2.7% from Sarawak and 71.4% from Sabah.

In Malaysia, malaria has remained a problem in Sabah and had reached epidemic proportions in 1975-1981 (Singh,1985). P.falciparum. is the predominant species (67.1%) followed by P. vivax (31.4%). P. malariae constitutes only about 0.4% of total cases in Malaysia . All cases of mixed infection were due to P. falciparum and P. vivax (Uma and Chee ,1988).

1.1.5 Future perspective

Control of falciparum malaria remains one of the world's

greatest health challenges. Generally, prevention of malaria can be tackled by chemotherapeutic (diagnosis and treatment of established infections), vector control, vaccination (under development) and health education.

Drug is an important element of malaria control, especially in areas where insecticides is less or not effective. Resistance of P. falciparum to 4-aminoquinolines has developed severe clinical forms of the disease . Therefore there is a continuing need for new , inexpensive drugs that are effective against resistant strains of P. falciparum , which are safe for field use and can be administered orally in a single dose.

Much attention has been directed to the development of new antimalarial drugs with less side effects especially drugs for severe malaria. The improvement of drug formulation and its efficacy for example ; halofantrine, artemisinin and its derivatives, benflumetol and etc.(WHO,1990) may fulfil the needs.

Combination of drugs may give effective protection against malaria. For example, the combination of pyrimethamine and sulphadoxine and chlorproguanil with dapsone were equally effective for treatment of falciparum malaria which is superior to chloroquine in non-pregnant and pregnant women (Keuter et al,1990). Chloroquine combination with calcium antagonists to "reverse" resistance has also been

demonstrated in vivo. These observations have stimulated several groups to consider using chloroquine in combination with calcium antagonists for the treatment of chloroquine-resistant malaria infections (WHO,1990).

Another approach which is still under development to eradicate malaria is by vaccination (Arnold,1990). Malaria vaccines should be used to solve problems in areas where malaria control has run into technical difficulties and in areas where control was not possible . Three main approaches to vaccination are the anti-sporozoites, anti-merozoite or anti-asexual blood stage and anti-gamete. The development of an anti-sporozoite vaccine against P. falciparum was based on defined polypeptides produced either by genetic engineering or chemical synthesis. Vaccines directed against asexual blood stages would probably curb mortality and morbidity and be particularly useful in areas with intensive malaria transmission (Arnold, 1990).

However, it was important to bear in mind that the clinical and epidemiological aspects of vaccine development are extremely complex. Guidelines have been established for such work by WHO but many questions regarding safety, frequency of vaccination, appropriate adjuvants, possible immunopathological sequelae and ultimate ability to afford protection against natural sporozoite challenge for different ages of people so far unanswered.

1.2 Review of analytical methods

In this thesis, works focus on biguanide antimalarials and therefore this section aims to give the review of the analytical work that have been done.

The earliest and most frequently used method of assay for this drug (Proguanil) in tissues and body fluids is by making the sample alkaline and extracting it into organic solvent, which is then acidified and extracted back into an aqueous phase (Spinks and Tottey, 1945 ; Maegraith et al, 1946 ;Schmidt et al , 1947 and Smith et al ,1961). The p-chlorophenylanaline is being released by autoclaving which caused a hydrolytic cleavage. The diazotisable amines is then determined by the Bratton-Marshall procedure. However this method allows quantification in the range of 1-50 ug/ml and is relatively insensitive. Smith et al (1961) ; Armstrong (1973) proposed another method which measured microbiologically the inhibition of growth of the folate-requiring bacterium Streptococcus facium (ATCC8043).

Generally, the spectrophotometric and bioassay methods have poor reproducibility in comparison with more precise physicochemical methods, and consequently they require a more complex assay design. Determination of in vivo bioavailability and pharmacokinetic profile in man require assay which is both sensitive and specific for the parent

drug or its major metabolites (Schwartz & de Silva, (1978). Blood concentrations of the parent drug may be hardly measurable due to the low dose administered, a high volume of distribution, rapid biotransformation and elimination of the drug.

Chromatographic techniques using High Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC) are more advantageous because they possess the required sensitivity and specificity. The review of various published analytical methods by these chromatographic techniques for the determination of proguanil, chlorproguanil and their major metabolites has been summarised in Table 1.

1.3 Review of pharmacokinetic and metabolism

Pharmacokinetics is a mathematical description of the process of absorption, distribution into and within tissues and elimination which relates the dose given, the concentration in the blood and the pharmacological response.

From the calculation of pharmacokinetic parameters, one can predict the drug concentrations as a function of time, dosage and route of administration. In addition, these parameters can be used to evaluate the effect of factors such as genetics, disease, other drugs and environmental conditions on drug disposition.

Table 1 : Summary of chromatographic analytical methods for proguanil and chlorproguanil in biological fluid.

Compound	Method of analysis	Extraction method	Column	Mobile phase	Limit of Detection	Author
Proguanil cycloguanil chlorophenyl-biguanide (CPB)	HPLC	Solid phase extraction	ODS-Hypersil 5um	Acetonitrile-10% w/v(1:1) SLS + Acetonitrile-water (50:50) + 0.175M phosphoric acid + 0.0125 NaH_2PO_4 (pH=1.5)	60ng/ml	Moody, Selkirk & Taylor (1980)
Proguanil cycloguanil	HPLC	Liquid-liquid extraction	Spherisorb nitrile 5um	Acetonitrile-methanol-water (9:2:89 v/v) + 0.05M Ammonium formate (pH=4.0)	10ng/ml	Kelly & Fletcher (1986)
Proguanil cycloguanil	HPLC	Liquid-liquid extraction	Supelco-NH_2 5um x 25cm x 46mm	Methanol-0.5% NH_3(25%) + Acetonitrile(1:1)	Proguanil 0.11ug/ml cycloguanil 0.05ug/ml	Bygbjerg & Flachs (1986)
Proguanil cycloguanil CPB	HPLC	Solid phase extraction	ODS-Hypersil 3um	Acetonitrile-10mM aqueous phosphate buffer (pH=2) + 200mM SLS (50:50)	Cycloguanil 0.5ng/ml CPB-0.5ng/ml proguanil 1.0ng/ml	Taylor *et al* (1987)
Chlorproguanil Chlorcyclo-guanil	HPLC	Liquid-liquid extraction	Spherisorb phenyl 5um	Acetonitrile-water containing 5mM 1-pentanesulphonic acid (35:65) (pH=3.8)	Chlorproguanil 5ng/ml chlorcycloguanil 10ng/ml	Edstein & Veenendal (1987)

Classical pharmacokinetics has developed sophisticated techniques for the analysis of drug conditions versus time. This has proven valuable in a variety of applications concerning drug action and usage. The main emphasis has been in the use of descriptive models (compartmental models). Although a few of the derived parameters may have direct physiological reality, for example blood clearance, the majority of these have no immediate association with the in vivo process. (Gibaldi & Perrier, 1982; Wagner 1976). Therefore there has been a tendency to overlook the fact that data from the experiment is the end of the interaction of a number of controlling biological processes. These limitations however, has led to the development of more physiologically oriented models of drug disposition which permit identification of the role and importance of certain in vivo processes. (Wilkinson & Shand, 1975, Rowland and Tozer, 1989).

Pharmacokinetic parameters are derived from time related changes in the concentration of drug and metabolite in plasma, whole blood or urine. Almost all of pharmacokinetic parameters in this thesis will be based on the analysis of plasma and metabolite concentration versus time data, using model independent formulae according to the method outlined below. This approach clearly discriminates between alterations in the rate of change in drug concentrations with time and also presents changes in drug concentration,

which usually occur exponentially, as linear processes.

The absorption of drug from the site of administration i.e. absorption phase may be followed by one or more distributive phases where drug concentrations decline due to distribution throughout the body. Finally there is the elimination phase, where drug concentrations decline due to irreversible loss of drug from the body.

Generally, the rates of the above processes obey first-order kinetics and are proportional to the concentration of drug in the body. However, there are exceptions and a number of compounds exhibit non-linear or zero order kinetics and can be described by the Michaelis-Menten equation (Gibaldi & Perrier, 1982). The methods used in this thesis to determine pharmacokinetic parameters apply only to drugs which obey linear, first order kinetics and may be divided into 2 categories: primary, pharmacokinetic parameters i.e, clearance and volume of distribution and secondary or derived parameters including elimination half life and area under the drug concentration versus time curve. Alterations in the values of primary parameters can be reflected by changes in the value of the secondary parameters.

1.3.1 Pharmacokinetic Principles

Pharmacokinetics describe the quantitative analysis of the

processes of drug absorption, distribution , elimination and excretion.

1.3.1.1 Drug absorption

Drug which are administered extravascularly and required to act systematically must first be absorbed. This involves the transfer of drug in solution across the separating biological membranes from the site of administration to the site of measurement. The rate and extent of the absorption process can have marked effects on the time of onset, intensity and duration of a pharmacological response produced by a drug. The mechanism of drug absorption is generally by passive diffusion of a drug down a concentration gradient from the gut to the blood stream with no expenditure of energy or by active transport which requires expenditure of energy, filtration through pores and by pinocytosis. Factors which influence the absorption of a drug include dissolution characteristics, molecular weight, pKa, lipophilicity, environmental pH, blood flow and gastric mobility.

The term bioavailability or systemic availability is used to describe the fraction of a drug which is transported from its site of administration to the site of measurement usually by systemic circulation.

1.3.1.2 Drug distribution

Following the entry into systemic circulation, a drug has to distribute throughout the body. Distribution is a reversible transfer process and it depends on the physicochemical characteristics of the drug, blood flow to various tissues and the binding of the drug to various tissue components and plasma proteins. Basic drugs may also bind to acute phase proteins such as α-1-acid glycoprotein. Once distribution is complete drug concentrations throughout the body are at equilibrium. Alterations in plasma drug concentration will then reflect similar changes in tissues concentrations throughout the body.

1.3.1.3 Drug elimination

Drug elimination is the irreversible loss of drug from the body by the process of metabolism and excretion. The main site of drug metabolism is primarily the liver and other tissues including kidney, skin, lung, blood, placenta and intestinal mucosa while the main routes of excretion are via elimination in bile or urine although there are instances when drugs have been excreted in sweat, saliva, expired air and natural milk (Gilbaldi, 1984).

The rate of drug metabolism usually varies individually

and usually is determined genetically but can be changed by environmental factors such as age, the environment, smoking, diet and alcohol. Biotransformation of many drugs produce metabolites that are less pharmacologically active (detoxification). However, certain metabolites have greater biological activity than the parent compound. Here, the parent compound is a 'prodrug' and the biotransformation product is a metabolite with a pharmacological action, for example, proguanil and chlorproguanil (Carrington, et al, 1951). The toxic effects of some drugs are caused by metabolites, for example paracetamol (Potter, et al, 1973).

The biotransformation of foreign compounds is catalysed by a wider variety of enzyme systems. The most important of which are the cytochrome P-450 mixed function oxygenase (Park, 1982). There are two types of biotransformation, phase 1 reactions are oxidation, reduction or hydrolytic processes while phase II reactions involve conjugation of drug to an endogenous molecule such as glucuronic acid, sulphate, amino acids or glutathione.

A large number of drug biotransformation have been shown to be catalysed by enzymes located on the smooth endoplasmic reticulum of hepatocytes. These enzymes have been named microsomal as they can be isolated from microsomes, which are small vesicles formed after mechanical disruption of the endoplasmic reticulum. Cyctochrome P-450 is a

haemoprotein which is the terminal oxidase involved in hydroxylation of numerous drugs and endogenous compounds, for example steroids. This haemoprotein exists in a number of forms of isozymes and the relative proportions of each are determined by factors such as species, genetics and environmental influences. (Brosen, 1990; Gonzalez, 1988, Guengerich & West, 1989 ; Boobis & Davis, 1984; Lu & West, 1980). Drug metabolising enzymes also have been isolated from other sources such as mitochondria, lysosomes, nuclei and cytosol.

1.3.1.4 Drug excretion

Excretion is the process by which drugs and their metabolites are removed from the body. The major pathways are the renal and biliary excretion, and also occur in other biological fluids.

In renal excretion, drugs and metabolites will undergo a glomerular filtration and will be forced across the renal tubular cell by an active transport mechanism against a concentration gradient. Certain compounds are subject to tubular reabsorption due to their physicochemical properties.

Drugs may be excreted by hepatic cells into the bile. They are occasionally excreted unchanged but more usually as conjugates (e.g. glucuronic acid, sulphate and glycine

conjugates). In general, polar metabolites secreted into the bile have relative molecular masses > 400. Drug conjugate complex is a good candidate for bilary excretion due to the increased molecular weight. Drug and its metabolites which are secreted into the bile and hence into the small intestine can undergo reabsorption into the systemic circulation and this process is termed enterohepatic recirculation (EHC). Thus, the EHC is a mechanism which prolongs the action of a drug.

1.3.2 The pharmacokinetic parameters

1.3.2.1. Elimination half-life ($t_{1/2}$)

Elimination half-life is defined as the time taken for plasma drug concentration and therefore the amount of drug in the body to fall by one-half after the attainment of distribution equilibrium. This value is a constant for a drug obeying first-order elimination kinetics and is independent of the amount of drug in the body.

Therefore, the amount of drug eliminated decreases in successive half-live. In theory, the drug can never be completely removed from the body, however, in practical terms, elimination is accepted as being complete after 5 half-lifes have elasped whereby 97% of this has been removed.

The elimination half-life can be determined from the

semi-log plot of drug concentration versus time and can be calculated as shown below:

$t_{1/2}$ = time for the concentration to decrease by half.

$t_{1/2} = \ln 2/k = 0.693/k$

1.3.2.2 Clearance (CL)

Drug clearances describe the relationship between the rate of drug elimination and drug concentration and is the most useful parameter for the evaluation of an elimination mechanism. Clearance is the volume of a biological fluid from which drugs is removed per unit time.

The value of clearance is dependent upon the site of measurement but independent of concentration for drugs which obey first-order elimination kinetics (i.e. clearance is constant). Total systemic clearance (CL) is the sum of all metabolic and excretory clearance processes which is the sum of all organ clearances. If the assumption is made that drug is completely metabolized by one organ e.g.: the liver, clearance is equal to the blood flow through the organ , therefore the equation can be written as :

CL = kVd

k = elimination rate constant

There is an inter-relationship between the clearance of a drugs, apparent volume of distribution and elimination half-life as follows:

$$CL = kVd$$

$$\text{Since } k = 0.693/t_{1/2}$$

$$CL = 0.693Vd/t_{1/2}$$

$$t_{1/2} = 0.693\ Vd/CL$$

$$\text{Hence, } CL = kVd$$

1.3.2.3 Apparent volume of distribution

This parameter relates the total amount of drug in the body to the measurement of drug concentration when distribution equilibrium has been achieved i.e.

$$Vd = Ab/Cp$$

Vd = Volume of distribution

Ab = the amount of drug in the body

Cp = the measured drug concentration

The value of this parameter rarely corresponds to a real physiological volume and is largely dependent upon the plasma and tissue binding of the drug, in addition to the physicochemical properties of the drug which determine the extent to which it will partition into biological membrane.

The volume of distribution can be calculated from plasma concentration versus time data as follows:

Vd = dose/AUC k

AUC = Area under curve

k = elimination rate constant

1.4 Review of drug available

Antimalarial drugs which are currently in use or have reached advance stages of clinical trials can be classified as follows:

(a) Cinchona alkaloids e.g. quinine and quinidine.

(b) 4-aminoquinolines e.g. chloroquine and amodiaquine.

(c) 8-aminoquinolines e.g. primaquine.

(d) Quinolinemethanols e.g. mefloquine.

(e) The antifolates comprising;

Dihydrofolate reductase inhibitors - e.g. proguanil, chlorproguanil , cycloguanil and pyrimethamine.

sulphonamides - e.g. sulphadoxine.

sulphones - e.g. dapsones.

(f) Antibiotics - e.g. tetracycline.

(g) The phenanthrenemethanols - e.g. halofantrine.

(h) The sesquiterpene lactones - e.g. artemisinin, artesunate, arteether and artemether.