EFFECTS OF ASCORBIC ACID ON THE PHARMACOKINETICS OF ARTESUNATE IN HEALTHY MALE VOLUNTEERS

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CHAPTER ONE

1.0 INTRODUCTION

1.1.0 BASIC CONCEPTS OF PHARMACOKINETICS

1.1.1 THE DYNAMICS OF PHARMACOKINETIC PROCESSES

Pharmacokinetics is the study of the process in which drugs are absorbed, distributed, metabolized and excreted from the body. It is concerned with obtaining the appropriate amount of drug at the receptor site for the appropriate length of time for that particular drug thus obtaining maximum therapeutic benefit with minimal side effects (Paxton, 1981).

Pharmacokinetics deals with the changes of drug concentration in the drug product and changes of concentration of drug and/or its metabolites in humans or animals following administration. This implies that the changes of drug concentration that occur in the different body fluids and tissues in the dynamic system of liberation, absorption, distribution, storage, metabolism and excretion, describing them as mathematical function of time and concentration of the drug. The application of pharmacokinetic principles to the therapeutic management of patients is clinical pharmacokinetics (Roland and Tozer, 1980).

The transfer of a drug from its site of administration to the blood, and the various steps involved in the distribution and elimination of the drug in the body may be depicted as shown in Fig. 1.1.

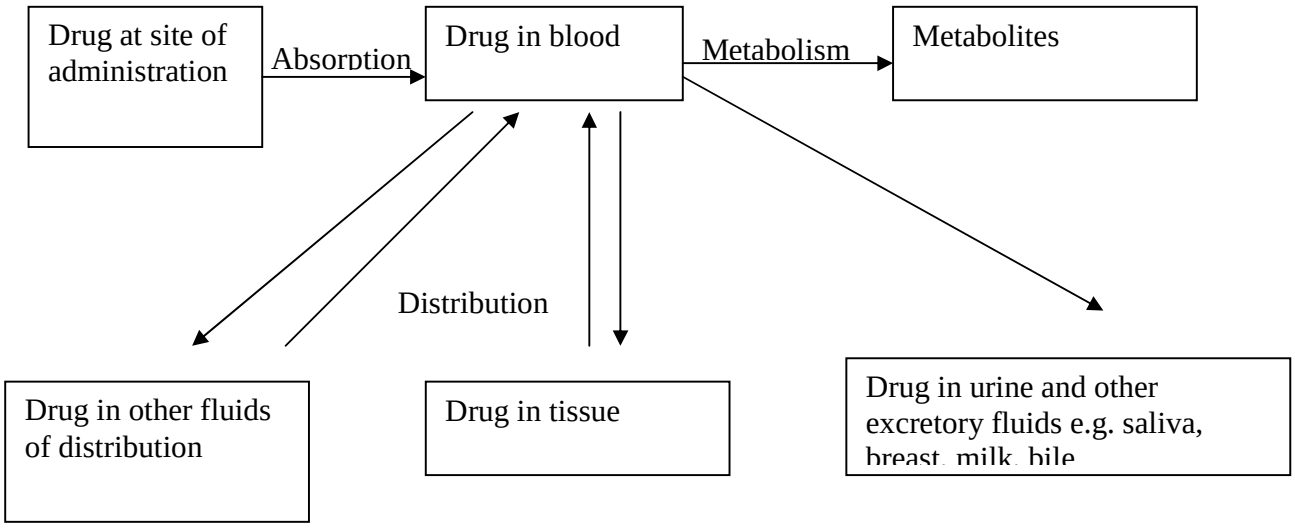


Fig. 1.1: Processes involved in the distribution and elimination of drug in the body.

The transfer of a drug from its absorption site to the blood, and various parts of the body is complex whereas the distribution of drug is a reversible process (see arrow), absorption and elimination of drug are irreversible. Attempts are made in pharmacokinetics to describe the dynamics of these processes in a system of compartment models with no apparent physiologic or anatomic reality.

PHARMACOKINETIC COMPARTMENT MODELS

1..1 A COMPARTMENT

A compartment model in pharmacokinetics is a hypothetical structure which can be used to characterize with reproducibility the behaviours and fate of a drug in

biological systems when given by certain routes of administration and in a particular dosage form.

A compartment is an entity which can be described by a definite volume and a concentration of drug. Compartment models are used to describe the behaviour of a drug in biological systems and could be classified into one, two, or multi-compartment models.

Firstly, drugs enter the system only via the central compartment and are eliminated only from that compartment. Secondly, reversible transfer occurs from central and peripheral compartments and thirdly, the exit of drugs from all compartments in the system is described by the first order kinetics which has unit of reciprocal time e.g.min^{-1},h^{-1}. Under this last assumption the rate at which a drug is removed from a compartment is directly proportional to the drug concentration in it. All the three assumptions are relatively valid for most drugs. The majorities of the drugs enters the body via the blood stream, and are eliminated from the blood stream by the liver and kidney. There are few exceptions which are primarily eliminated by biotransformation and if given in high enough doses, result in saturation of the metabolic enzyme system. The elimination then becomes zero-order i.e constant and independent of drug concentration (Paxton, 1981).

1.2.2 ONE-COMPARTMENT MODEL

This is the simplest model which depicts the body as a single homogenous unit in which the drug entering the body distributes instantly between the blood and the body fluids or tissues (i.e. the exchange of drug between the plasma and tissue proceeds rapidly compared with the rate of elimination). Here, the whole body is considered mathematically as a single compartment. This model is useful for the pharmacokinetic analysis of blood, plasma or serum concentration. It is also useful for analysis of salivary and urinary excretion for drugs which are rapidly distributed between plasma and other body fluids and tissues upon entry into the systemic circulation.

A compartment model assumes that any change that occurs in the plasma quantitatively reflects changes occurring in tissue drug levels. Assuming instantaneous distribution after an intravenous injection into this model (Fig. 1.2) the concentration (C_o) in the plasma immediately after injection is equal to the dose(D) divided by the volume of this compartment (Vd).

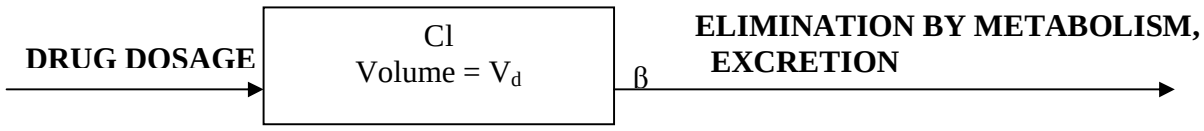


Fig. 1.2: One-Compartment Model Kinetics

This apparent volume of distribution (V_d) of the drug is not a true volume, but that volume into which all the drug in the body would appear to be distributed to achieve a concentration the same as that in the plasma. Elimination follows a first order kinetic after instantaneous distribution which means that a constant fraction of drug is eliminated per unit time. Accepting first order kinetics between drug concentration and time, the decline in drug concentration may be expressed mathematically as:

$$\frac{dc}{dt} = -\beta C_t$$

where C_t = concentration

β = rate of constant of elimination

Integration and conversion to logarithm to base 10 give

$$\text{Log } C_t = \log C_o \quad \frac{\beta t}{2.303}$$

1.2.3 TWO-COMPARTMENT MODEL

The plasma concentration time curve of a drug is often not a straight line on a semi-logarithm plot. If distribution of the drug is so slow that it cannot be disregarded, a model must then be considered which contains a central compartment and at least one other peripheral compartment (Fig. 1.3).

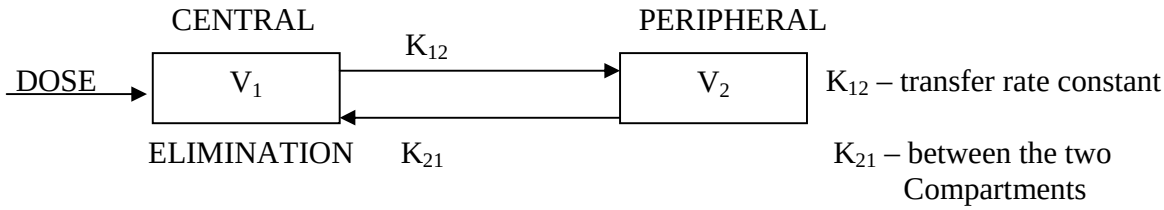


Fig. 1.3: Two-Compartment Model Kinetics

Although, these compartments lack physical and anatomical reality, for many drugs, the central compartment corresponds to the plasma or blood volume, together with extracellular fluid of highly perfused tissues such as heart, liver, kidneys and endocrine glands. Drugs distributed within a few minutes through this compartment and equilibrium between plasma and tissue is rapidly established. The peripheral compartments are then formed by less perfuse tissues such as skin, muscle or adipose tissues in which drugs enter more slowly. The combined effect of two compartments gives rise to a bi-phasic Curve on i.v injection with two distinct linear portions when drawn on a semi log scale

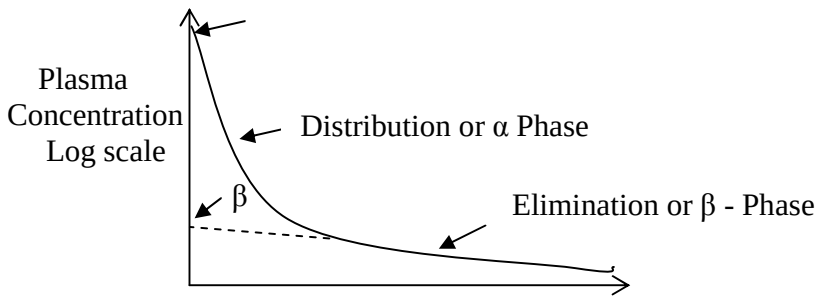


Fig. 1.4: Plasma log – concentration time curve for a two compartment model

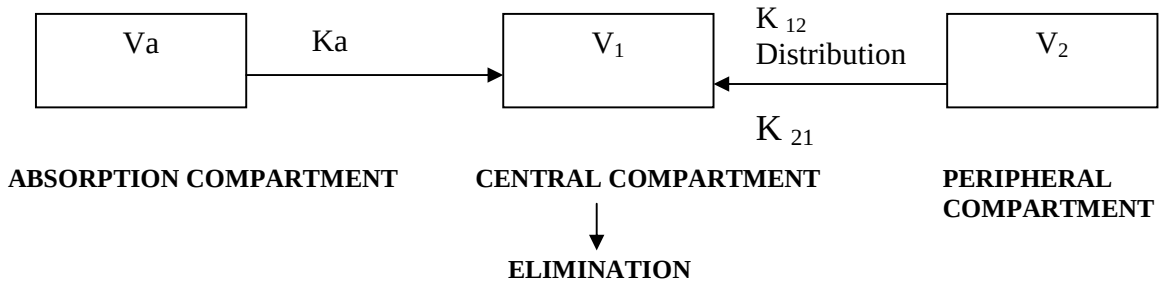
Although drug distribution is slow, it is usually much faster than elimination. Thus, the initial rapid fall in concentration (known as the α-distribution phase) mainly represents the relatively rapid process of drug distribution from central to peripheral compartment.

Once distribution is complete, the curve enters the relatively slow or elimination phase during which drug disappearance is determined mainly by irreversible elimination from the central compartment. The two compartment model may be expanded to contain additional compartment which can be described mathematically as the sum of as many individual exponential functions as there are relevant compartments (multi-compartment models). In reality, a maximum of three-compartments is allowed in assay techniques (Paxton, 1981).

1.2.4 THREE-COMPARTMENT MODEL

This is a design or a modified model for oral dosing in which an additional compartment is incorporated to represent the volume (Va) from which absorption occurs at a first order rate – Fig. 1.5.

In this model, it is assumed that the entire dose is rapidly introduced into the site of absorption, from which it is absorbed into the central compartment. This compartment model also explains other administration routes such as intramuscular or subcutaneous which involves a preliminary absorptive phase.



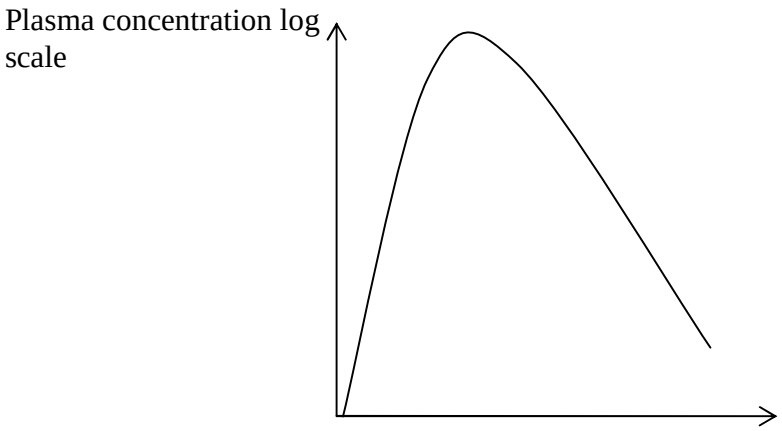


Fig. 1.5: A three compartment model which includes a compartment for absorption – a typical log concentration time curve, after a single oral dose.

1.3.0 PHARMACOKINETIC PARAMETERS

The following pharmacokinetic parameters (in alphabetical order) shall be determined in this study.

1.3.1 ABSORPTION HALF-LIFE t ½ ab or t ½α

This is the time taken for half the dose of the administered drug to be absorbed. $t_{1/2}$ ab is affected by all the factors which influence drug absorption such as drug formulation, pH of the stomach or Pka of drug etc. t½ ab is of no

significance in intravenously administered drugs and is calculated manually through the method of residuals on a semi-logarithm graph of plasma drug concentration time or with the computer.

1.3.2 ABSORPTION RATE CONSTANT (Ka)

Absorption rate constant is a proportionality constant relating to the rate of drug absorption in the body. This is a first order kinetics where the rate of absorption is directly proportional to the drug concentration at the absorption site per unit time (e.g. min^{-1} or h^{-1}).

i.e. Rate of absorption ka.Aa - - - (1)

Where Aa is amount of drug remaining to be absorbed. In this case, the driving force for absorption is the difference between the concentration of the diffusing drug at the absorption site (Ca), and that unbound in the arterial blood perfusing the absorptive membrane (Cu), thus

Rate of absorption = P.A. (Ca-Cu) --- (2)

Assuming that the volume of fluid at the absorptive site (Va) remains relatively constant, then the distribution yields

Rate of absorption ‘ $\frac{[P.A].A}{[Va]}$ ------(3)

Thus, the permeability constant of the drug, the surface area and volume of fluid at the absorptive site determine the value of Ka. As with other first order

processes, absorption is characterized by t½, and is related to t½ exponentially through the equation:

$$Ka = \frac{0.693}{t½} \text{ i.e. } \frac{\text{Natural Log2}}{t½}$$

1.3.3 APPARENT VOLUME OF DISTRIBUTION (V_d)

This is defined as that volume in which the amount of drug in the body would need/appear to be uniformly distributed to provide the observed plasma concentrations. It is used to relate the plasma concentration of a drug to the dose administered (if no elimination has occurred) or to the amount of drug in the body. V_d is inversely proportional to the blood drug level. A large value for V_d implies wide distribution or extensive tissue uptake or both. After oral drug administration, V_d can be determined from the equation:

$$V_d = \frac{F.D}{KeAUC} \text{ or } V_d = \frac{Cl}{Ke}$$

Where, AUC = Area under curve

D = Dose

F = Bioavailable fraction

Cl = Systemic clearance

Ke = Eliminating rate constant.

1.3.4 AREA UNDER CURVE (AUC)

Area under the curve is the integral of drug blood level over time from zero to infinity (o-∞) and is a measure of the quantity of drug absorbed (bioavailable) in the body.

AUC is a major determinant of the therapeutic efficacy. The methods of calculating area under the curve are discussed by various authors. These methods include planimetry, cut and weigh, trapezoidal rule and computer integrated application. AUC is important for calculating important pharmacokinetic parameters such as oral bioavailability, total plasma clearance of drug and apparent volume of distribution. It can be calculated from the equation:

$AUC = C_o\ dt$

Where C_o = concentration of drug at zero time

dt = time difference and unit is ug/ml.h

1.3.5 BIOAVAILABILITY

Bioavailability is the term used to indicate measurement of the relative amount of administered dose that reaches the systemic circulation. Bioavailability therefore deals with the rate and extent of drug absorption. The more rapid the absorption, the shorter the onset of action and the greater the intensity of pharmacological response (Rowland,1995).

For orally administered drug bioavailability is determined by how rapidly they are cleared by the liver. Thus, drugs that have high first pass effect are usually poorly bioavailable e.g. lidocaine, propanalol, morphine, etc (Laurence and Bennett, 1984).

Dosage forms of a drug from different manufacturers and even different lots of preparations from a single manufacturer sometimes differ in their bioavailability. Such differences are seen in oral dosage forms of poorly soluble and slowly absorbed drugs. They result from differences in crystal form, particle size and other physio-chemical properties of the drug that are not rigidly controlled in formulation including the coating materials. These factors affect the disintegration of the dosage form and dissolution of the drug and hence the rate and extent of drug absorption (Ansel, 1981). The absolute bioavailability of an oral dose can only be determined when an intravenous (i.v) study with the same dose in the same subject is performed.

Bioavailability is designed F and

$$F = \frac{\text{Amount of drug reaching systemic circulation after oral dose}}{\text{Amount of drug reaching systemic circulation after intravenous dose}}$$

By definition, F ranges between zero and one. In order for two drugs forms to be bioequivalent, the amount of drug absorbed from each must be the same, and the rate of absorption from each drug product must be comparable. The absorption

rate can be obtained by comparing the time of occurrence of peak plasma concentration (T_{max}) after a single dose.

The extent of absorption can be determined by two methods:

1. By determination of the total area under the drug concentration (in plasma) versus time curve (AUC)
2. By determination of the total amount of unchanged drug excreted in the urine.

In both cases, these results are taken in comparison with result obtained with a standard preparation. This standard may be an intravenous injection or an orally administered aqueous solution or even another drug product accepted as a standard.

For practical purposes, in the determination of bioavailability, the AUC is used. The AUC in the plasma concentration versus time Curve has the unit of concentration time e.g. mg/ml.h and can be determined by several methods (see 1.3.4).(Rowland, 1995).

1.3.6 CONCENTRATION AT ZERO TIME (Co)

Co is an extrapolated value, which is an estimate of the drug concentration multiplied by the volume of distribution (V_d) to give the dose of administered drug

i.e. Dose of administered drug = V_d . Co

For practical purposes, Co is estimated by extrapolating the straight line of the elimination phase of plasma drug concentration time graph to zero time.

1.3.7 ELIMINATION HALF LIFE ($t_{½}\ \beta$)

The elimination half life ($t_{½}\ \beta$) is the time taken for half the amount of drug present in the plasma to be eliminated either by excretion or metabolism or both. In practical terms, it is the time taken for its plasma concentration to be reduced by half or 50% of its original value (Tripathi. , 2003).

Elimination plasma half-life can be determined for both one and two compartment models from the terminal data of the plasma log-concentration time plot. It is calculated by dividing 0.693 (natural logarithm of 2) by the calculated slope of each exponential phase of multi-phasic blood level versus time curve.

i.e. $t_{½}\ \beta = \frac{\text{In } 2}{\text{Ke}}$

The $t_{½}\beta$ is a poor index of drug elimination process and dosage requirements of individual patients as it depends not only on the clearance (elimination processes) but also on the volume through which the drug is distributed. However, it is a useful parameter in the determination of suitable dosage intervals and time required to attain the steady state during constant intravenous or chronic oral therapy.

1.3.8 ELIMINATION RATE CONSTANT (Ke)

The elimination rate constant may simply be regarded as the fractional rate of drug removal. It characterizes the elimination process. The proportion constant is known as first order rate constant with dimensions of time^{-1} Ke that is calculated by the equation:

$$Ke = \frac{F\,D}{V_d AUC} \text{ or } \frac{Cl}{V_d}$$

Where F is fraction of administered drug absorbed

D is dose of administered drug.

V_d is the volume of distribution of drug

AUC is a measure of the quantity of drug absorbed (bioavailable)in the body.

Cl is systemic clearance

Ke is also experimentally related to half – life ($t_{½\beta}$) by the equation

$$Ke = \frac{0.693}{t_{½\,\beta}}$$

The relationship between absorption rate constant (Ka) and elimination rate constant (Ke) determines the rate of change of drug concentration in the body as:

Rate of absorption – Rate of elimination = Rate of change of drug concentration in the body.

$$Ka.Aa - Ke.A_b = \frac{d.A_b}{dt}$$

Where

Aa is amount of drug remaining to be absorbed

A_b is amount of drug absorbed in the body

$d.A_b$ is the total quantity of drug in the body

dt is the total time.

This means that when Ka>ke, blood drug levels will rise and will continue to do so, until an equilibrium is reached at Ka = Ke when the rise will stop at the peak level. The peak level will remain constant as long as this equilibrium is maintained, but drug level will start to decline once Ke>Ka until the whole drug is eliminated from the body.

1.3.9 LAG TIME (t_{lag})

The lag time (t_{lag}) is the time interval between the administration of a drug product (dosage form) and first appearance of the drug in systemic circulation. It can only be observed upon extravascular (ev) route of administration e.g. oral, intramuscular, rectal, topical etc and is affected by factors affecting absorption. It is calculated by computer integrated application or manually through the method of residuals on a semi-logarithm plasma drug concentration time graph.(Rowland, 1995).

1.3.10 MAXIMUM DRUG CONCENTRATION (C_{max})

This is the peak blood level of the administered drug per unit time and is reached when the rate of absorption equals the rate of elimination (ka = ke) C_{max} is instantaneous for intravenously administered drugs and it is important in estimating drug dosages during therapy. It is easily read off on a semi-logarithm plasma concentration time graph. The C_{max} is proportional to dose. Thus, if the dose is doubled the maximal blood concentration is also doubled (Rowland, 1995).

1.3.11 SYSTEMIC OR PLASMA CLEARANCE (Cl)

This is defined as the volume of plasma from which a drug is totally and irreversibly removed per unit time. Total plasma clearance of a drug is the sum total of individual clearances of the drug by the various organs of drug elimination, namely, exhalation by the lungs, hepatic metabolism, renal and fecal excretion systemic or plasma clearance is a direct index of drug elimination and is influenced by factors which affect the organs of elimination such as blood supply, diseases etc. It is expressed in volume per unit time e.g. ml/h (Rowland, 1995).

Cl is expressed by: $Cl = \frac{F.D}{AUC} = \frac{\text{Absorbed dose of drug}}{\text{total bioavailable drug eliminated}}$

Where

F = fraction of drug absorbed (bioavailable)
D = Dose of drug administered.

Clearance is also related to elimination rate constant (ke) and the apparent volume of distribution (Vd) by the equation:

$Cl = ke. V_d$

1.3.12 TIME TO ATTAIN MAXIMUM CONCENTRATION (t_{max})

This is the time taken by the administered drug to attain its highest concentration (peak level). Like C_{max}, t_{max}, depends on the equilibrium between the rate of absorption and the rate of elimination, and is very important in all extravascular routes of administration. t_{max} is used in the determination of dosage and dosage intervals. It is used to determine the absorption rate of any drug. The time t_{max} after which the blood concentration becomes maximal is concentration – independent given a particular drug and method of administration, the maximal blood level will occur at the same time whether the dose is large or small. (Rowland, 1995).

1.4.0 VARIABILITY IN PARAMETER VALUES

Many of the variables affecting pharmacokinetic parameters have been recognized and can be taken into account.(International Biopharmaceutical Association,2005).

1.4.1 AGE AND WEIGHT

For some drugs, changes in pharmacokinetics with age and weight are well established. In children and young people, renal function appears to correlate well with body surface areas. Thus, for drugs primarily eliminated unchanged by renal excretion, clearance varies with age according to the change in surface area. In person over age 20, renal function decreases about 10%/year. For neonates and young infants, both renal and hepatic functions are not fully developed and no generalization, except for the occurrence of rapid change, can be made.(International Biopharmaceutical Association ,2005).

1.4.2 DISEASE

Renal function impairment: The renal clearance of most drugs appears to vary directly with creatinine clearance, regardless of the renal disease present. The change in total clearance depends upon the distribution of the kidneys to total elimination. Thus, (total) clearance is expected to be proportional to renal function (creatinine clearance) for drugs solely excreted unchanged and not to be affected for drug eliminated by metabolism.

In acute renal failure plasma artemether levels after intramuscular administration were increased, this is possibly related to the reduced volume of distribution as renal elimination of these drugs is negligible (Michiel, 1999).

Hepatic disease: produces changes in metabolic clearance, but good correlates or predictors of the changes are unavailable. Dramatically reduced drug metabolism has been associated with hepatic cirrhosis. Reduced plasma protein binding is often observed in this disease because of lowered plasma albumin. Acute hepatitis, with elevated serum enzymes, is usually not associated with altered drug metabolism. Heart failure, pneumonia, hyperthyroidism and many other diseases also alter the pharmacokinetics of drugs. (International Biopharmaceutical Association, 2005).

Although Artemisinin is mainly metabolized by hepatic transformation it has been found that liver disease like liver cirrhosis did not influence bioavailability nor delay the elimination (Peter, 1999).

1.4.3 DRUG INTERACTION

Many interactions can cause changes in pharmacokinetic parameter values and therefore in drug response. Most of these interactions are graded, and the extent of the interaction depends upon the concentrations of both of the interacting drugs.

For instance, in the interaction study of artemether-lumenfantrine and quinine in healthy subjects, the plasma level of artemether and dihydroartemisinin appeared to be lower when co-artemether was given in combination with quinine compared to co-artemether alone (Gilbert,2003).

1.4.4 DOSE AND TIME DEPENDENCE

In some instances, the value of the pharmacokinetic parameters changes with dose administered, concentration in plasma or time e.g. a decreased bioavailability of griseofulvin as the dose is increased, a disproportionate increase in the steady state plasma phenytoin concentration or increasing its dosing rate and a decrease in plasma carbamazepine concentration during its chronic use. The decreased bioavailability of griseofulvin is due to the drug's low solubility in the GI tract. Phenytoin shows a concentration (dose) dependency because the metabolizing enzymes have a limited capacity to eliminate the drug and the usual rate of administration approaches the maximum rate of metabolism.(International Biopharmaceutical Association,2005).

Artemisinin exhibited both dose-time dependence. In multiple dose studies with artemisinin, in both healthy subjects and patients peak plasma artemisinin concentration on day six were reduced to only 20% when compared to those on day one, whereas the half life was unchanged. It was suggested that auto-induction of enzymes in the liver or gut was responsible for this time dependent increase in first pass effect (Michiel, 1999).

1.4.5 PREGNANCY

Little information is currently available on effects of pregnancy on the pharmacokinetics of drugs in humans. As pregnancy advances, important physiological changes occur in the haemodynamics, the relative volume of body water components, the hormonal milieu, the concentrations of plasma proteins and endogenous compound such as non-esterified fatty acids, all of ,which are known to influence drug disposition and kinetics.

Steroid production is significantly increased in pregnancy, and since progesterone is known to stimulate hepatic mucosal enzyme activity, this could lead to greater rate of hepatic metabolism for some drugs e.g. antipyrine and diazepan (Kraumar et al., 1979).

No abnormalities and contraindications were found in pregnant women during the second or third trimester of pregnancy in the treatment of uncomplicated malaria (WHO, 2001).

1.4.6 ENVIRONMENTAL FACTORS

Environmental factors also contribute to individual variations in drug metabolism. Factors such as diet content of certain macromolecules (protein or carbohydrate) and the mode of preparation, such as charcoal broiling of meat, have been shown to markedly influence drug biotransformation in man.

Certain vegetables e.g. cabbages and brussel sprouts have also been implicated in the stimulation of the metabolism of drugs. Other well known inducers of the hepatic drug metabolizing system include chlorinated hydrocarbon insecticides such as Dichlorodiphenyltrichloroethane (DDT) and polycyclic aromatic hydrocarbons (PAH) such as 3-4, benzypyrene these are products of incomplete combustion and are spread in man's environment, occurring in particularly high concentrations in tobacco and polluted city air.(Vestal,1995).

1.4.7 GENETIC INFLUENCE

Pharmacogenetics is the study of hereditary variations in drug response. Hereditary differences in the amount of structure of a key enzyme may result in significant variation in the rate of drug metabolism in man. Where distinguishable differences in a given characteristic exist, it is called polymorphism. The mode of inheritance is either monogenic or polygenic depending upon whether it is transmitted by a gene at a single locus or by genes at multiple loci.

Another way of describing the two distinct types is continuous or discontinuous. The former is more common and arises when the inherited characteristics are controlled by several genes of small effect (i.e. multifactoral inheritance).

The distribution of the given characteristic will show a continuous variation and will yield a unimodal or gaussian distribution curve. On the other hand, where a characteristic depends on single gene of major effects, a bimodal or even trimodal distribution in the population may be observed. This monofactoral inheritance is much less common and has so far been restricted to a few specific examples, the best known of which is the hepatic enzyme N-acetyl-transferase. This enzyme catalyses the transfer of an acetyl group to a number of drugs including isoniazid, sulphadimidine, dapsone and hydrallazine. Measurement of plasma concentration of isoniazid for example, six hours after oral administration, resulted in a bimodal distribution histogram. The subpopulation groups were designated “rapid” and “slow” acetylators which vary in different races.

Other inheritable factors recognized in the use of drugs include defective plasma cholinesterase resulting in prolonged action of suxamethonium and the haemolytic activity on red blood cells by some antimalarial drugs e.g. primaquine due to deficiency of gluco-6-phosphate dehydrogenase.(Vessel,1980).

1.5.0 DRUG INTERACTION

A drug interaction occurs when either the pharmacokinetics or the pharmacodynamics of one drug is altered by another.

A therapeutic drug interaction occurs when one drug significantly alters the degree of response to another drug when two or more drugs are taken in combination. The outcome of concurrent drug administration may diminish therapeutic efficacy or increase toxicity of one or more of the administered drugs.(Paula,2001).

Drug interactions occur or arise frequently because multiple drug therapy is the rule than the exception. In this study, drug interactions are classified on the basis of whether the pharmacokinetics of a drug is altered.

1.5.1 PHARMACOKINETIC DRUG INTERACTION

In this case, either the pharmacokinetics of one or both of the drugs administered is altered i.e. the absorption, distribution, metabolism or excretion of one drug or both drugs is affected by being administered concurrently.

Distinction between pharmacodynamic and pharmacokinetic drug interaction is best made by relating response to unbound concentration of the pharmacologically active species. Change in the unbound concentration – response curve implies a pharmacokinetic drug interaction (Tozer et al., 1980).

Pharmacokinetic drug interaction can be evaluated quantitatively by reference to changes in the pharmacokinetic parameters of the test drug (Absorption t½ α, elimination t½β, AUC etc).

1.5.2 DRUG ABSORPTION INTERACTION

Interaction during the absorption phase results in either an increase or decrease in the relative rate of absorption or an increase or decrease in the amount (extent) of drug absorbed.

With respect to rate of absorption, a drug may be absorbed so slowly such that even though it is completely absorbed it may never reach effective serum concentration, for example, procainamide change in rate of absorption will not alter the area under the concentration time curve.

Other examples are acetaminophen and propantheline (propantheline decrease the absorption rate of acetaminophen, acetaminophen and metoclopramide) metoclopramide increase rate of absorption of Acetaminopen.

On the other hand, changes in extent of absorption have different implications; there is alteration in the AU $C_{0-\infty}$. Alteration in extent of absorption is exemplified thus – digoxin and propantheline. Propantheline enhance availability of slowly dissolving digoxin).

The absorption of drugs from the gastrointestinal tracts is a complex process that depends on many physiochemical and physiological factors (Levine, 1970).

An enumeration of a number of mechanisms are as follows: pH effect on dissolution and ionization, changes in gastric emptying and GI motility, formation of complexes, ion pairs and chelates, interference with active transport etc.

Daily induced changes in pH of gastrointestinal fluid may have complex and unpredictable effect on the absorption of other drugs taken at the same time. The stimulatory effect of alkali in gastric emptying may explain the apparent increase in propantheline absorption caused by bicarbonate sodium (Chaput des Saintang and Hexheimer, 1973).

H_2 receptor antagonist will raise pH of the gastric fluid and therefore increase rate of absorption of drugs that are absorbed majorly in the small intestine. There are several mechanisms by which bioavailability of one may be affected by the other. Induced changes in the gastric intestinal environment such as altered gut motility, blood flow, effect of ionization, pH, physiochemical interaction such as chelating; complexation, adsorption and dissolution. Physiological factors such as enzymes and tyramine in food.

Ranitidine an H_2 – receptor antagonist has been reported to delay gastric emptying time with concurrent administration therefore decreasing delivery of drug which eventually affects absorption of other drugs (Scarpignato *et al.*, 1982).

1.5.3 DRUG DISTRIBUTION INTERACTION

Drugs are reversibly bound to tissue, plasma proteins and receptors. The ratio of free drug to bound drug at each of this site depends on the properties of the drug, disease condition in patients (e.g. uraemia, hypo-proteinaemia) and plasma protein concentration and concentration of the drug.

Drug distribution interaction leads to alteration in volume of distribution due to alteration in tissue and plasma protein binding. It should be noted from the foregoing that competitive interaction for same binding site between two or more interacting drugs is implied because binding might not always result from displacement.

Two conditions must be met for substantial and clinically significant displacement in plasma to take place. First, the drug must be bound to protein (unbound concentration must be low $\leq$ 10%). Secondly, the displacer must have high affinity for the same binding site. Rantidine and promethazine are 15% and 93% protein bound respectively. Therefore displacement interaction between both drugs will not be clinically significant.(Hansten,1994).

1.5.4 DRUG METABOLISM INTERACTION

Drug metabolism implies chemical transformation of a drug within a biological system leading to an alteration in its bioactivity. Implicit in the above

definition is the fact that drug metabolism may result in more active/toxic or less active/toxic or inactive/non-toxic species.

Drugs are transformed by a variety of pathways. They are adequately grouped into phases viz, phase I and phase II respectively. Phase I reactions result into formation of more polar metabolite from parent drug by introducing or unmasking a functional group. The different pathways may involve oxidation and reductive process e.g. aromatic C-oxidation (hydroxylation), N-dealkylation, N-oxidation etc. However, a phase II reaction may take place for phase I metabolites that are not rapidly eliminated. This subsequent metabolic process involves conjugation with an endogenous metabolic substrate such as glucuronic acid, sulfuric acid, acetic acid and glycine to form highly polar conjugates.

Two microsomal enzymes play a key role in metabolism. The first is the flavour protein NADPH. Cytochrome P450 reductase. The second is the haemoprotein called Cytochrome P450 that serves as terminal oxidation. Concurrent administration of drugs can lead to an increase in rate of metabolism of the other drug by induction of the enzymes responsible for metabolism.(Kastrup,2000).

1. 5. 5 ENZYME INHIBITION

Enzyme inhibition is an extremely common mechanism behind the interaction of two drugs such interactions often result in exaggerated and prolonged responses with an increased risk of toxicity. Most inhibitory interaction involves the hepatic mono-oxygenase enzyme .A number of drugs have the capacity to inhibit these enzymes, usually by competitive binding to cytochrome P450 to form a stable complex which prevents cytochrome P450 enzyme system to have access to other drugs.

Imidazole containing drugs such as cimetidine and ketoconazole bind tightly to the haem iron of cytochrome p450 and reduce the metabolism of endogenous substrates e.g. testosterone or other co-administered drugs through competitive inhibition. This leads to prolongation of the effects of other drugs. Other enzyme inhibitors are chloramphenicol (metabolite), spironolactone etc.(Brodie,1998).

1. 5. 6 ENZYME INDUCTION

Reduced effect of many drugs has been shown to be due to the induction of metabolic enzymes. Induction results in an acceleration of metabolism and subsequent decrease in the pharmacological action of both the inducer and co-administered drug.

In the case of drug metabolically transformed to reactive metabolites, induction may exacerbate metabolite induced toxicity. Example of induction is a co-administration of barbiturates with warfarin. Barbiturates increase metabolism of warfarin .(Brodie1998).

1.5.7 DRUG EXCRETION INTERACTION

Drugs are eliminated by urinary excretion through three mechanisms viz, glomerula filteration, tubular reabsorption, tubular secretion. Interaction may occur by competition for tubular transport. For example, the effect of probenecid on increasing serum levels of penicillin is well known. However, concurrent use of probenecid and sulphinpyrazone does not alter the individual uric acid and clearance obtained with each drug alone. Also half lives of some drugs can be greatly changed by changes in urinary pH induced by alkalinizers such as sodium bicarbonate or acidifiers such as ammonium chloride (Kastrup, 2000).Examples are salicylates and amphetamine respectively.

1.6.0 SALIVA (AS BIOANALYTICAL SAMPLE)

The biological fluids that are most commonly analyzed are whole blood, plasma (or serum), and urine. Less common fluids requiring analysis include bile, sweat, milk and saliva. Saliva may be considered particularly useful where it can be shown to accurately reflect the concentration in blood as it is a non-invasive method.

Saliva is a colourless transparent/translucent, somewhat viscid material of low viscosity. Its attraction for bioanalysts is that it is relatively free of interfering substances, is easily extractable by organic solvents and is thought to reflect the levels of non-protein bound drug in the blood. For non-ionized drugs such as steroids, the drug should readily cross from plasma to saliva and this would represent a non-invasive method of monitoring.

Normal subjects may produce up to 2 litres of saliva in 24 hours with a more or less continuous flow of 15ml/h between meals. Collection of a reasonable sample in a reasonable time is tedious for normal subjects but larger samples can be obtained by stimulation of glands, by chewing an inert material such as paraffin, parafilm, rubber bands, pieces of Teflon (Dawes and Macpherson, 1997) will usually elicit a flow of 1to3ml/min.

Stimulated saliva is also reported to be more consistent with its pH value (pH 7.0 to 7.8). The composition of saliva compares well with those of blood, plasma and serum. Authors reporting of the analytical methods for determining drug in saliva did not seem to have experienced any particular problem in adapting existing methods. For other fluids such as plasma or serum, minor problems have been noted with adsorption of phenytoin to mucoid proteins of saliva and the occasional adsorption of drugs to the stimulation material.

Most drugs appear to enter saliva by passive diffusion process which is characterized by the transfer of drug molecules down a concentration gradient with no expenditure of energy. The rate of diffusion drug is a function of the concentration gradient, the surface area over which the transfer occurs, the thickness of the membrane, and a diffusion constant that depends on the physio-chemical properties of each drug (Paxton, 1979).

Analysis that used saliva for quantification of drugs is ultraviolet spectrophoto meter, high perfomance liquid chromatography. Examples of drugs that are analyzed using saliva include mexiltene, chloroquine, promethazine, artemisinin and its derivatives, paracetamol, etc.

1.7.0 AIMS OF THE STUDY

The study has the following specific aims:

(1) Determine pharmacokinetic parameters of artesunate.

(2) To evaluate pharmacokinetic parameters of artesunate when co-administered with Vit. C

1.8.0 RESEARCH QUESTIONS

(1) What are the pharmacokinetic parameters of artesunate when administered alone?

(2) What are the pharmacokinetic parameters of artesunate when co-administered with ascorbic acid?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1.0 GENERAL

2.1.1 NAME: ARTESUNATE (ARTEMISININ)

2.1.2 HISTORY AND CHEMISTRY

Artemisinin (ginghaosu) is the antimalarial principle isolated by Chinese scientist in 1972 from the plant *Artemisia annua* L, a sesequisterpene lactone (empirical) formula ($C_{15}H_{22}0_5$) bearing a peroxy group. Artemisinin is hydrophobic and passes biological membranes easily.

Artemisinin is the active ingredient in ginghao, a Chinese herbal tea that has been used for 150 years to treat malaria and now has been found to grow in other parts of the world too. It is prepared as an infusion of dried leaves.

Derivatives of artemisinin, artesunate, artheether and artelinate, artemisinin and these compounds are quickly converted to their plasma metabolite, dihydroartemisinin which is the chemical with the antimlaria activity. The oil soluble derivatives are arthemether, and arteether, and the more water soluble dervivative sodium artesunate and artelinic acid. These derivatives have more potent blood schizonticidal actively than the parent compound, and the most rapidly effective antimalaria drug known. They are used for treatment of severe and

uncomplicated malaria. They are not hypnozonticidal but gametocytocidal activity has been observed. They are adversely rapid and most patients show clinical improvement within 1-3 days after treatment. Artemisinin and its synthetic derivatives are available for treatment of falciparum malaria infection (Meshnick *et al.,* 1996).

Artemisinin has a short elimination half life, a rapid onset of action. A major drawback with artemisinin and its derivatives is high recrudescence rate (within 2-3 weeks after monotherapy) (Li., 1989). It has proved difficult in field studies to determine whether the relapse malaria episodes are due to new infections or the persistence of the previous one. It has been suggested that the occurrence of recrudescence may partly be due to the decreasing artemisinin concentration towards the end of treatment (Ashton *et al.,* 1998). Artemisinin, artemether and artesunate have been used in China for treatment of uncomplicated malaria and increasingly in the rest of South East Asia for the last decade.

In general, oral formulations of these drugs are rapidly and incompletely absorbed and their bioavailability is low (Bethel *et al.,* 1997). There is good evidence that they undergo extensive first pass metabolism in the liver. Both artesunate and artemether are rapidly transformed into dihydroartemisinin so that the metabolite is generally present at higher levels, than the parent compound (Teja et *al.,* 1996).

Although dihydroartemisinin is the most active derivative in a molar basis each of the parent drugs are active in the low nM range and are known to achieve levels much higher than the minimium inhibitory concentrations in the plasma. In spite of the rapid clearance and extent of transformation, the parent drugs may contribute a significant proportion of the antimalarial effect in the blood (Hien and White, 1993, Tierkule *et al.*, 1993).

Despite the limitation of many of the reported clinical studies, a constant observation is that artemisinin derivatives produce faster relief of clinical symptoms and clearance of parasite from the blood than other antimalaria drug (Hien and White, 1993). In about 90% of the patients given these drugs, the fever resolved and the parasitaemia cleared within 48 hours of treatment. It has been estimated that they reduce the parasite biomass by a factor of approximately 10^4 fresh 35-48 hours asexual cycle of the parasite and by a factor of 10^6-10^8 over a 3-day course of treatment (White, 1997). Despite their short elimination half-life (1.6.-2.6 hours) artemisinin drugs are effective when given daily.

The problems with the artemisinin drugs is that when they are used over short periods of less than five days, clearance of parasitaemia from the blood is only temporary in up to 50% of patients. This has been attributed to the short time that it takes for artemisinin drugs to be eliminated from the body. Higher efficacy can be obtained by 5 and especially 7-day regimens but this is associated with

reduced compliance in outpatients. Artemisinin are reportedly well tolerated in adult and children, no serious adverse effects have been reported following their clinical use. They are reliably effective against multi-drug resistant falciparum malaria when combined with mefloquine. It improves parasite clearance compared with either drug alone.

In order to increase compliance, attempts have been made in Thailand to reduce the required days of drug administration (Bunneg *et al.,* 1991, Norten *et al.,* 1994). A one-day course of 10mg/kg of either artemisinin ,artesunate together with mefloquine (10-15 mg base/kg) resulted in recrudescence rate of 15-20%. A single dose of 300mg arthemether or artesunate followed by 25mg mefloquine base/kg in divided doses gave cure rate of >95%.

Artesunate and arthemether have been shown to clear parasitaemia more effectively than chloroquine and sulphadoxine/pyrimethamine in Nigeria and Tanzania (Alin *et al.,* 1996, Ezedinachi, 1999). Artemisinin and its derivative have a significant effect on gametocytogenesis. Laboratory studies have shown that they prevent gametocyte development by their action on the ring stages and on the early stage (State I – III) gametocytes (Kumar and Rhang, 1990). The observations have been confirmed by field studies (Chen *et al.,* 1994).

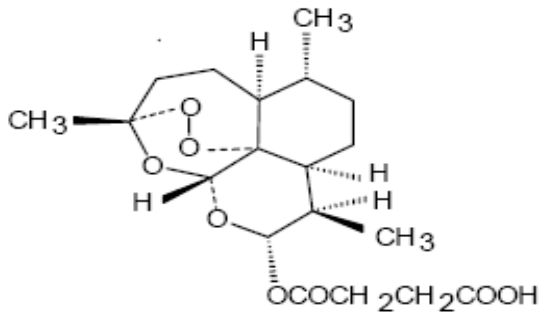


Figure 2.1: Structural formulae of artesunate (Dihydroartemisinin-12-α-succinate)

2.2.0 MECHANISM OF ACTION

In- vitro studies have suggested an uptake of artemisinin by both healthy and malaria infected red blood cells. It is known that artemisinin binds to haem, either in haemoglobin (inside red blood cells) or haemozoin (stored haem within the malaria parasites). Through an iron-mediated cleavage of the peroxide bridge artemisinin free radicals are formed. These free radicals are destructive to different parasite membrane, including mitochondria, rough endoplasmic reticulum and plasma membranes, thereby killing them (Gordi et al, 2001).

It is believed that the haem in – rich internal environment of the parasites is one of the reasons for the selective toxicity of artemisinin toward the malaria parasites as haemin has been shown to interact with the compound (Gordi et al, 2001).

2.3.0 TOXICITY

Well-documented clinical uses of artemisinin and derivatives have shown few insignificant side effects but findings in rat studies are causing controversy. Intramuscular arteether at 25mg/kg/day resulted in rats brainstem pathology with damage to the auditory nuclei. Parenteral artemether in the treatment of cerebral malaria in Gambian children showed a prolonged recovery from coma and post-treatment convulsions than the same treatment with quinine. Also in Vietnamese adults, prolonged recovery was noted, but no increase in mortality and neurological behaviour afterwards (Happner and Ballou, 1998). Others are transient heart block, transient decrease in blood neutrophils, and brief episodes of fever.

Despite several reports on neurotoxic effects of artemether (Brawer *et al.*, 1994), this family of compounds has proven to be extremely well tolerated in clinical practice (Dayan *et al.,* 1998). The neurotoxicity seen in animals after high doses of these compounds has not been reported in human (Ambroise, 1991). However, a longer time for recovery from coma in cerebral malaria patients has been observed in those treated with intramuscular artemether compared to intramuscular quinine.

2.4.0 PHARMACOKINETICS

Artemisinin has a quick onset of action thereby stopping the parasites from developing and cyto adherence is not recorded. Drug disposition of artemisinin after oral administration has been determined, using a one compartment model with separate pharmacokinetic estimated for children and adults. The population estimates for artemisinin clearance and distribution volume respectively were 432 L/kg^{-1} and 37.92L/kg^{-1} for children with inter-subject variability collectively for both age group of 45% and 10.4% respectively. The oral bioavailability was estimated to decrease from day 1 to day 5 by a factor of 6.9, a value found to be similar for children and adults (Sidhu *et al.*, 1998).

Half life of artemisinin is 4 hours and artemether 4-11 hours (Batty *et al.*, 1998). Findings like this advocated the dosing of artemisinin to children according to a standard dose.

CytochromeP-450 enzymes 2B6 with some possible contribution of CYP 3A4 and CYP2A6 have been suggested to metabolize the compound. Rectal administration of artemisinin resulted in lower plasma concentrations of the drug compared to oral doses, although no significant difference was found in the elimination half lives between the two administration routes.

Fraction bound artemisinin to plasma proteins averages around 80-85%. Artemisinin exhibits time and dose dependent kinetics in both healthy volunteers

and malaria patients. These include trends for a possible saturable first pass metabolism and decreased plasma concentration upon repeated administration of the substance. The capillary and saliva sampling had been suggested as promising replacements for various sampling in pharmacokinetic studies of artemisinin (Gordi et al, 2001).

2.5.0 EMPIRICAL LITERATURE REVIEW

Pre-clinical studies have consistently shown that artemisinin and its derivatives do not exhibit mutagenic or teratogenic activity, but all of these drugs caused foetal resorption in rodent at relatively low doses of 1/200 – 1/400 of the LD50> 10mg/kg when given after sixth day of gestation. Reports on the use of these drugs during pregnancy are limited (WHO, 2001). However, malaria can be particularly hazardous during pregnancy. Artemisinin and its derivatives are therefore the drugs of choice for severe malaria and can be used for treatment of uncomplicated malaria during the second and third trimester of pregnancy in areas of multiple drug resistance. Their use in the first trimester is not recommended (WHO, 2001).

Between 1992-1995, an assessment of gametocytaemia in over 5000 adults and children in western Thailand near the border with Myanmar showed that treatment of ,uncomplicated falciparum malaria with either artesunate or artemether

greatly reduced gametocytaemia (Price *et al.,* 1996) compared with mefloquine. The artemisin derivatives reduces gametocyte rate by a factor of eight in primary infections and by a factor of 18.5 in recrudescent infections. Artemisinin pharmacokinetic in healthy adult after 250, 500 and 1000mg sample oral dose shows that inter-individual variability in artemisinin pharmacokinetic was large with parameter coefficient of variation (CV) typically between 50 – 70%. The parameter with the smallest variability was elimination half- life (CV) approximately equal to 30-40% (Ashton *et al.,* 1998).

Analysis of variance indicated also a large intra-subject variability, CV of 2-4% for the dose normalized area under the plasma concentration time curve (AUC) dose. The pharmacokinetic results suggested artemisinin to be subject to high pre-systemic extraction. Artemisinin half -life could not predict the extent of in vivo exposure to the drugs, there being no correlation between half- life and oral clearance. Artemisinin oral plasma clearance is about 400 Lh^{-1} (Ashton et al., 1998) exhibiting a slight decrease with dose although the effect was weak, thus results from studies using different artemisinin doses range can be compared without the complication of disproportionate changes in drug exposure with varying dose levels. Half-life appeared to increase with dose. An observed period effect in the analysis of variance has tentatively associated with time-dependency in artemisinin in plasma concentration determined at various time points after 500 and 1000mg

doses, but less after 250mg doses. This may show a tentative approach to assess the systemic exposure of the patients to artemisinin from the determination of artemisinin plasma concentration, one or two plasma samples only artemisinin was well tolerated with no apparent dose or final dependent effects on blood plasma, heart rate or body temperature.

Peter (1999) investigated whether the clearance of artemisinin in patients with liver cirrhosis is different from healthy volunteers; a pharmacokinetic study was performed in male Vietnamese patients with hepatitis B induced cirrhosis of liver who received 500mg of artemisinin orally. The results were compared to those found in a previous study in healthy subject. The mean (± S.D) area under the concentration time curve was 2365 (±176.2)ng/ml, the mean (± S.D) clearance, 385 (±30.3). The elimination half life was (±2.3) h estimated by non-linear regression using a one-compartment first order elimination model. The mean (± S.D) absorption time was 2.55 (± 0.8h) These results were not different from the results of healthy subjects and shows that liver disease has no effect on the availability and clearance of oral artemisinin, indicating that artemisinin has an intermediate hepatic extraction ratio and that there is no significant first pass effect.

2.6.0 ASCORBIC ACID (VITAMIN C)

Vitamin C (Ascorbic acid) is obtained from citrus fruits, tomatoes, strawberries, cabbage, greens and potatoes, oranges and lemon juice are outstanding source containing approximately 0.5mg/ml (2.8m/M). Ascorbic acid is a powerful reducing agent and functions in a number of biochemical reactions mostly involving oxidation. Ascorbic acid is usually administered orally, readily absorbed from the intestine via an energy dependent process. The recommended dietary allowance (RDA) for vitamin C is 60mg daily, based on threshold urinary excretion of vitamin C and preventing the vitamin C deficiency disease scurvy with a margin of safety(*Levine,* 1996). Threshold urinary excretion of vitamin C was reported at 60mg daily dose (Levine, 1996).

Complete plasma saturation was found to occur at 1000mg daily. Neutrophils, monocytes and lymptocytes saturated 100mg daily and contained concentration at 14 fold higher than plasma. Bioavailability was found to be complete at 200mg of vitamin C as a single dose (Food and Nutrition Board, Institute of Medicine, 1994). The daily intake of vitamin C is 60mg (current adult RDA) and achieved plasma concentration of 0.8mg/dl (45 m UDA) and the body store is about 1500mg of intake raise beyond 200mg daily the body store tends to level at 2mg/dl (110 mum). It is present in the plasma, leukocytes and in the tissue.

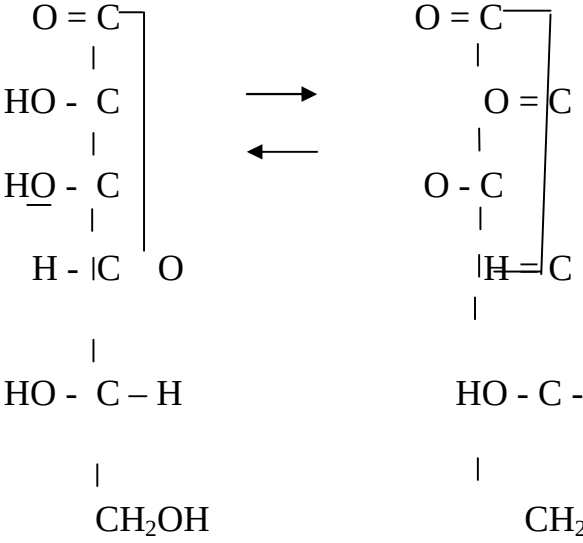
The white blood cells of healthy adult have concentration of about 27µg of ascorbic acid per 10^8 cells (Vallance, 1990).

L-ascorbic is converted to oxalate in man and excreted as dehydroascorbate in urine. One of the metabolites of ascorbic acid is ascorbic acid -2-sulphate and is greatly in excess of the physiological requirements. High dose of vitamin C acidify the urine thus affecting the elimination of weak bases. Acidic urine may cause precipitation of urate and cysteine stone in the urinary tract.

2.7.0 CHEMISTRY OF ASCORBIC ACID (VITAMIN C)

Ascorbic acid is a six carbon ketolactone structurally related to glucose and other hexoses. It is reversibly oxidized in the body to dehydro-ascorbic acid. The latter compound possesses full vitamin C activity. The structural formulas of ascorbic acid and dehydro-ascorbic acid are as follows:

Figure 2.2: Structural formula of Ascorbic acid



Ascorbic acid Dehydro-ascorbic acid

Ascorbic acid has an optically active carbon atom and antiscorbic activity resides almost totally in the L-isomer. Another isomer erythorbic acid (D-isoascorbic acid, D-araboscorbic acid has very weak anti-ascorbutic action but has been used to present nitrosamine formulation from nitrites in cured meats such as bacon. The reason for the lack of a stronger antiscorbutic action of erythrobic acid is probably the incapacity of the tissue to retain it in the quantified form that ascorbic acid is stored. One consequences of the facile oxidation of ascorbic acid is the readiness with which it can be destroyed by exposure to air, especially on an alkaline medium and it appears it is present as a catalyst.

2.8.0 ABSORPTION, RATE AND EXCRETION

Ascorbic acid is readily absorbed from the intestine and absorption of dietary ascorbate is nearly complete (Kallner et al., 1999). When vitamin C is given as a single oral dose, absorption decreases from 75% at 1 gram to 2 at 5 grams. Ascorbic acid present in the plasma and ubiquitously distributed in the cells of the body. Concentration of the vitamin in leukocytes are sometimes taken to represent dose in tissue are less susceptible to depletion, than in the plasma. The white blood cells of healthy adults have concentrations of about 27µg of ascorbic acid per 10^8 cells. It should be noted that the amount of ascorbic acid in leukocytes may be inversely related to their number and estimates of ascorbate acid status may

be falsely low in patients with leukocytosis in whom white cell ascorbic acid is measured (Robert et al, 2000).

Adequate ingestion of ascorbic acid is associated with concentration over 0.5 mg/dl (28µm) whereas concentration of 0.15 mg/dl (8.5µM) are seen in individuals with frank scurvy and the total body store of the vitamin at this time is approximately 300mg. When the intake of ascorbate is raised, the concentration in plasma also increases at first linearly. The concentration deviates from linearly at about 200mg/day and achieves saturation at 1000mg/day. When 60mg of vitamin C is consumed per day (the current adult RDA), the concentration in plasma reaches about 0.8 mg/dl (45µm) and the body store is found to be 1500mg.

If intake is raised beyond 200mg daily, the body store tend to level off at 2500mg and the concentration in plasma at 2mg/dl (100µm). The renal threshold for ascorbic acid is about 1.5mg/dl of plasma(85µm), and increasing amounts of ingested ascorbic acid are excreted when the daily intake exceed 100mg.Urinary excretion of oxalate and urate increase when daily ascorbic acid intakes approach 1000mg(Levine,1996). Ascorbate is oxidized to Co_2 in rats and guinea pigs, but considerably less conversion can be detected in man. One route of metabolism of vitamin C in man involves its conversion to oxalate and eventual excretion in the urine as dehydroascorbate is presumably an intermediate. Ascorbic acid -2- sulfate which also has been identified as a metabolite of vitamin C in human urine.

2.9.0 HUMAN REQUIREMENT

The daily intake of ascorbic acid must equal the amount that is excreted and destroyed by oxidation. Healthy adult human subjects lose 3 to 4% of their body store daily. To maintain a body store of 1500mg of ascorbic acid or more in an adult man, it would thus be necessary to absorb approximately 60mg daily.

South Africa runners have been observed to require 200 to 250mg of vitamin C daily to maintain a plasma concentration of 0.7 mg/dl (43µm) (Goodman and Gilman, 2000). Concentration of ascorbate in plasma is lowered by the use of cigarettes and oral contraceptive agents but the significance of those changes is unclear, requirement can increase in certain diseases and particularly infectious diseases and also following surgery (Levine, 1993).

2.10.0 DRUG INTERACTION

Mefloquine has no effect on plasma concentration profiles of oral artesunate. There are similar data after the administration of dihydroartemisinin supporting the conclusion that mefloquine does not influence the absorption, distribution, metabolism or excretion of artesunate.

From the available literature, artesunate is unlikely to have significant impact on the absorption and distribution of mefloquine. Some observations on C_{max} values made by Karbwang et al (Karbwang, 1994) could mean that artesunate

decreases the bioavailability of mefloquine. Data from other studies do not support this conclusion, but rather suggest that artesunate and mefloquine can be given together with confidence (Davis, 2006).

Regarding metabolism and excretion, available evidence suggests that the terminal elimination half time for these drugs is similar whether they are given alone and in combination (Davis, 2006). Furthermore, in vitro studies have shown that artesunate does not alter mefloquine metabolism by human liver microsomes.

No pharmacokinetic interaction with other antimalarial drugs of importance has been identified. There is a theoretical risk that a pharmacodynamic interaction with desfferroxamine might attenuate the antimalarial activity of artesunate. Studies were conducted to evaluate the antimalarial potential of clindamycin in combination with dihydroartemisinin in continuously cultured and in freshly isolated *Plasmodium falciparum* histidine rich protein II synthesis

Interaction analysis revealed a synergistic additive mode of interaction at various concentration ratio in all continuously cultured parasites at the 50% effective concentration (EC 50) level. In fresh *P. falciparum* isolation, a fixed clindamycin – dihydroartermisinin combination exhibited additive activity at the EC50 and EC90 levels (Ramharter , 2003).

In-vitro data confirm that Azithromycin in combination with artemisinin derivatives or quinine exerts additive to synergistic interaction (Horald ., 2007).

The interaction between atovaquone and dihydroartemisinin showed antagonistic effect against the atovaquone sensitive strain tested.

The quinolines and the artemisinin peroxide act antagonistically in -vitro against *P. falciparum* in culture. They both rely to some degrees on the generation of haem within the parasite in order to exert their full effects (Taramelli ., 2005).

Studies were carried out between artemisone and chloroquine, atovaquone or pyrimethamine with 1`antagonistic effect was found between these drugs but synergistic effect occurs between artemisone and mefloquine or lumenfantrin carried out in vitro. In this study, the interaction of artemisinin with lumenfantrin was slightly synergistic against the drug resistant KI strain slight antagonism between artemisone or artesunate and tefenoquine against 307 and KI strains, which contrasts with synergistic effect between tafenoquine and artemisinin against multi-drug resistant isolates reported previously.

Similar contrasting results were found with artemisone and artesunate plus amodiaquine, in which antagonism was observed, despite a synergistic interaction between artemisinin and amodiaquine. These differences may be related to different drug ratios used or varying degrees of drug susceptibility of *P. falciparum* strains or isolates used in each study.

The synergistic effects observed in- vitro with artemisone and mefloquine were reported in- vivo and were similar to those previously reported for artesunate

in combination with mefloquine. The combination of artemisone with chloroquine and with clindamycin had synergistic effect in- vivo, particularly with chloroquine against the chloroquine resistant *P yoelic* N.S and less apparent with clindamycin in *P berghei* N.Y (Nagamune, . K, 2007).

The pharmacokinetic and tolerability of artesunate and amodiaquine alone and in combination in (15) healthy volunteers were carried out by (Orrell *et al.*, 2008) and it was observed that the mean AUC was lower for dihydroartermisinin (ratio 67%) (95% CL 51-88%) p = 0.008) and desethylamodiaquine (ratio 65%, 95%, Cl 46-90%), p = 0.0015) when compared with monotherapy. The total drug exposure to both drug were reduced significantly.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1.0 DRUGS

3.1.1 Artesunate – 50mg tablet

Vami's artesunate manufactured by

TUYIL PHARMACEUTICAL INDUSTRIAL LIMITED

22, Stadium Road, Ilorin, Kwara State, Nigeria.

Batch No. TVA 307

MFG Date: September, 2007

Exp. Date: August, 2010

3.1.2 Vitamin C (Ascorbic Acid) 100mg tablet.

Vitamin C Tablet; (Em – Vit. C)® 100mg

Manufactured by:

EMZOR PHARMACEUTICAL INDUSTRIES LIMITED

Plot 3C Block A, Aswani Market Rd, Isolo, Lagos, Nigeria.

Batch No: BN 2218M

MFG. Date: 07 May, 2007

Exp. Date: 10 May, 2010

3.2.0 EQUIPMENT

3.2.1. Analytical balance (Mettler AE 240)

3.2.2. Ultraviolet spectrophotometer 6505 U.V/Vis, Jenway

3.2.3. Vortex Mixer (Gallenkamp)

3.2.4. Centrifuge machine (Gallenkamp)

3.2.5. Stop watch/clocks

3.2.6. Test tubes (pyrex)

3.2.7. Sample bottles of various sizes

3.2.8. Centrifuge tubes

3.2.9. Test tube clamps

3.3.0 REAGENTS

3.3.1. N-hexane (BDH Chemicals Ltd Poole England)

3.3.2. Sodium hydroxide pellets (BDH Chemicals, Poole England)

3.3.3. Hydrochloric Acid (Analar Grade BDH Chemicals, Poole, England)

3.3.4. Ethyl acetate (Analar grade BDH Chemical Poole, England).

3.3.5. Distilled water.

3.3.6. Methanol (Riedel-de Haen,Germany).

3.4.0 DEVELOPMENT OF ANALYTICAL METHOD.

3.4.1 Selection of Analytical Wavelength.

Analytical method was developed using Jenway 6505 U.V/VIS spectrophotometer by selection of Analytical Wavelength i.e preparing 40µg/ml stock solution of the standard Artesunate in different solvents water, ethyl acetate, n-hexane and scan through U.V region of 400-200nm to obtain λmax with the various solvents to determine the highest solubility. λmax 300nm of sample with n-hexane shows sharp peak which was then selected as a workable Wavelength.

3.5.0 PRECISION AND PERCENTAGE EXTRACTION RECOVERY OF THE ANALYTICAL METHODS

The precision of the analytical method was carried out for both within –day and between- day analysis by preparing 40µg/ml and running five times and the mean concentration and% C.V was calculated.

3.6.0 EXTRACTION METHOD

Extraction method was adopted and modified from (Orish et al., 2001). 2ml of saliva sample was added to 6ml of n-hexane. The mixture was rotard mixed for 30 seconds and centrifuged for 5 minutes at 2000rpm. The supernatant fluid (containing free drug) was collected in a sample bottle and absorbance was taken using U.V spectrophotometer at 300nm. Also Percentage extraction recovery was

determined to provide efficiency of the extraction method and reproducibility using % extraction recovery procedure below.

PERCENTAGE EXTRACTION RECOVERY

$$= \frac{\text{Amount recovered from sample concentration µg/ml}}{\text{Amount recovered from 0.1N HCL}} \times \frac{100}{1}$$

3.7.0 CALIBRATION CURVE

Calibration curve was prepared using blank saliva sparked with different concentrations of standard artesunate powder prepared by serial dilution of 10, 20, 30, 40, 50 µg/ml in methanol. Thus 2ml of blank saliva and 1ml of each prepared concentration of 10µg/ml, 20, 30, 40, 50µg/ml; with 6ml of n-hexane was rotardmixed for 30 seconds and centrifuged for 5 minutes at 2000 rpm. Absorbance was then measured at 300nm.

3.8.0 VALIDATION OF THE CALIBRATION CURVE

Validation of the calibration curve was determined by preparing concentrations outside those of the curve i.e. 15, 25, 35, 45 µg/ml to ascertain their fitness in the graph.

3.9.0 IN VIVO STUDY

3.9.1 Ethical Approval

Ethical approval was obtained from Research Ethical Committee of Ahmadu Bello University Teaching Hospital, Zaria and before taking part in the research, consent was obtained from all the twelve volunteers.

3.9.2 Study Design (Protocol of the Study)

Twelve (12) healthy young adult male volunteers with average age of 26±4 years and average weight of 66.25kg±5 took part in the study. Prior to participation; they were all properly screened and certified healthy. No drugs including alcohol and cigarettes were allowed two weeks before their participation in the study. The study was designed with single oral dose (200mg) of Artesunate in two stages cross-over. In stage1 Artesunate (200mg) alone was given and saliva sample was collected at 0, 15, 30, 45mins, 1, 2, 3, 4, 5, 6hrs.

After a washout period of two weeks stage II commenced with co-administration of Artesunate (200mg) and vitamin C (200mg) after which saliva samples were collected again at the same time interval written above. Sample collected was then stored in the refrigerator at -4^{O}C before analysis.

3.10.0 DATA HANDLING AND STATISTICAL ANALYSIS

Absorbance values were converted to concentration by calibration curve and pharmacokinetic parameters were generated by residual method, and AUC by trapezoidal rule and values were compared using student t-test and $p<0.05$ was considered to be statistically significant.

CHAPTER FOUR

4.0.0 RESULTS

4.1.0 PRECISION OF ANALYTICAL METHOD.

The precision of analytical method using spectrophotometeric assay of artesunate in saliva was estimated with 40µg/ml for both with-day and between-day determination. The coefficient of variation (cv) for within-day was 2.24% and1.75% for between-day. The low values of the coefficient of variation indicated the efficiency of the analytical method (Table 4.1).

Table 4.1: Precision of analytical method used for spectrophotometric assay of artesunate in saliva.

PRECISION	CONCENTRATION µg/ml	CV%	n
Within-day Precision of Artesunate.	40.00	2.24	5
Between-day Precision of Artesunate.	40.00	1.75	5

4.2.0 PERCENTAGE EXTRACTION RECOVERY OF ANALYTICAL METHOD USED FOR SPECTROPHOTOMETRIC ASSAY OF ARTESUNATE IN SALIVA

The percentage extraction recovery of the analytical method was established. Percentage extraction recovery was found to be within the range of 92-94% and this indicates high efficiency in the extraction procedure employed.

Table 4.2: Percentage Extraction Recovery of Analytical Method Used for Spectrophotometric Assay of Artesunate in Saliva.

SAMPLE	CONCENTRATIONµg/ml	CV% ±SD	N
Artesunate	34.84	94%±1.06	5
	34.01	92%±1.06	5

4.3.0 CALIBRATION CURVE FOR ARTESUNATE IN SALIVA USING A SPECTROPHOTOMETRIC ASSAY.

A graph of calibration curve was constructed with values obtained below. a straight line graph was obtained after a plot of absorbance(nm) versus concentration µg/ml. The calibration curve was linear with Pearson's correlation coefficient, r=0.98.The validation of the curve was assured using concentration outside those of the curve. This is to ascertain their fitness in the graph.

Table 4.3: Absorbance Reading of Calibration Standard of Artesunate in Saliva

CONCENTRATION µg/ml	ABSORBANCE nm
10.0	0.647
20.0	0.700
30.0	1.045
40.0	1.400
50.0	1.619

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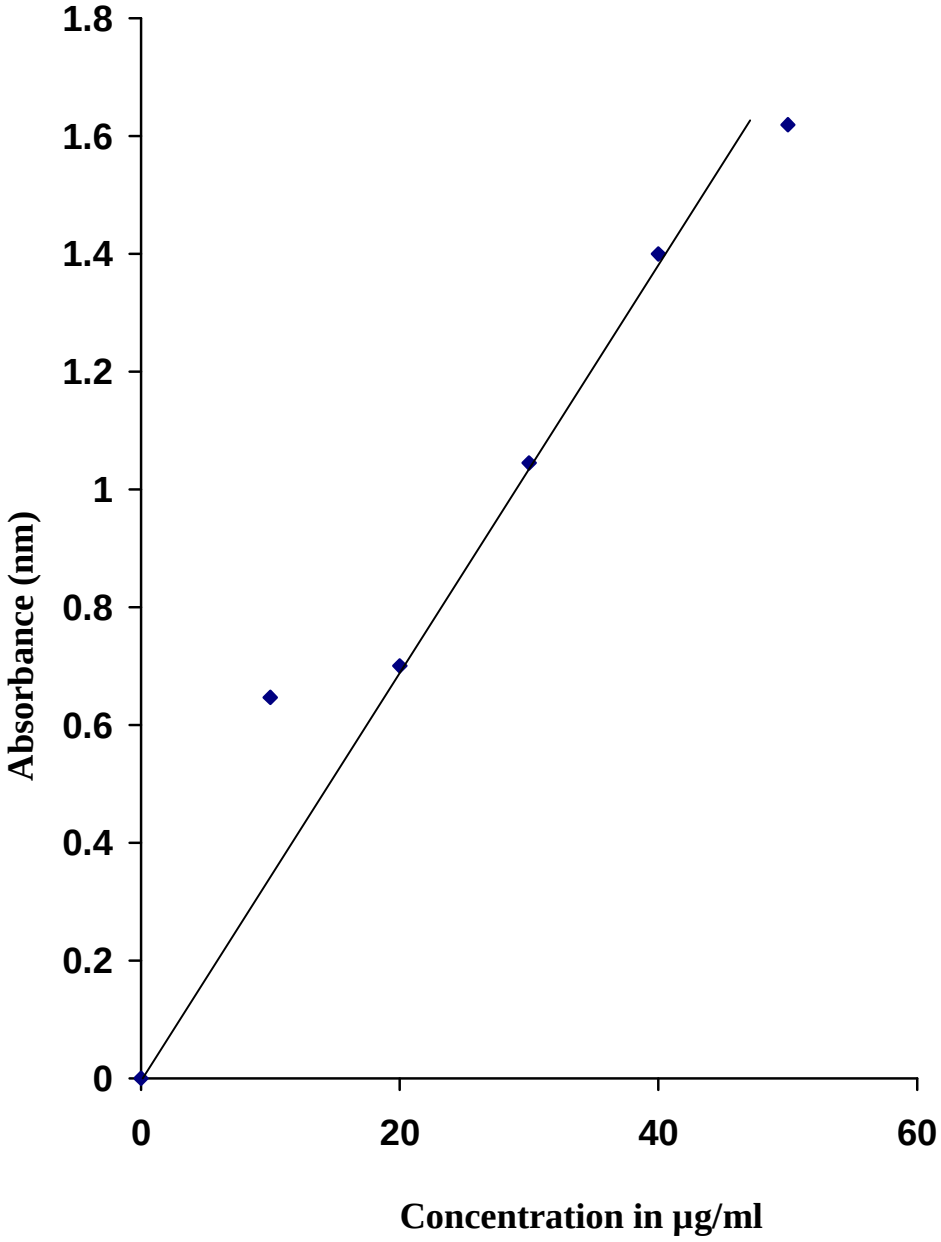

Fig. 4.1: Calibration Curve for Artesunate in Saliva using a Spectrophotometric Assay

Table 4.4 shows the mean salivary concentration of artesunate when administered alone (stage1).The data showed that artesunate levels peaked at 49.65µg/ml at 30 minutes and by 6 hours the level was down to 18.67µg/ml. When co- administered with 200mg of ascorbic acid the resulting mean concentration is depicted in Table 4.5. It can be observed that there were considerable reductions in mean salivary concentrations of artesunate. The peak concentration even though occurring at 1 hour was only 13.00µg/ml.

When the levels were compared statistically the reduction was significant at all the times measured ($p < 0.05$). This is shown in Table 4.6.

Table 4.4: Mean Salivary Concentration of Artesunate in 12 Healthy Volunteers after the oral Administration of 200 mg Tablets.

TIME(minutes)	MEAN CONCENTRATION μg/ml±SEM
0.00	0.00±0.00
15.0	18.93±0.37
30.0	49.65±0.56
45.0	45.56±0.56
60.0	34.84±0.32
120.0	34.01±0.30
180.0	30.28±0.18
240.0	26.38±0.17
300.0	23.39±0.2
360.0	18.67±0.37

Table 4.5: Mean Salivary Concentration of Artesunate in 12 Healthy Volunteers after the Concurrent oral Administration of Artesunate (200mg) and Ascorbic Acid (200mg).

TIME(minutes)	MEAN CONCENTRATION μg/ml±SEM
0.00	0.00±0.00
15.0	11.22±0.21
30.0	11.08±0.19
45.0	9.78±0.13
60.0	13.06±0.26
120.0	9.51±0.10
180.0	7.73±0.12
240.0	9.89±0.13
300.0	7.03±0.16
360.0	7.02±0.16

Table 4.6: Statistical Analysis of Mean Salivary Concentrations of Artesunate when Administered alone and when Co-Administered with Ascorbic acid.

	CONCENTRATION mglml		
TIME (minute)	ARTESUNATE ALONE	ARTESUNATE + ASCORBIC ACID	P VALUES
0.00	0.00±0.00	0.00±0.00	P>0.05
15.00	18.93±0.37	11.22±0.21	P<0.05
30.0	49.65±0.56	11.08±0.19	P<0.05
45.0	45.56±0.56	9.78±0.13	P<0.05
60.0	34.84±0.32	13.06±0.26	P<0.05
120.0	34.01±0.30	9.51±0.10	P<0.05
180.0	30.28±0.18	7.73±0.12	P<0.05
240.0	26.38±0.17	9.89±0.13	P<0.05
300.0	23.34±0.20	7.03±0.16	P<0.05
360.0	18.07±0.37	7.02±0.16	P<0.05

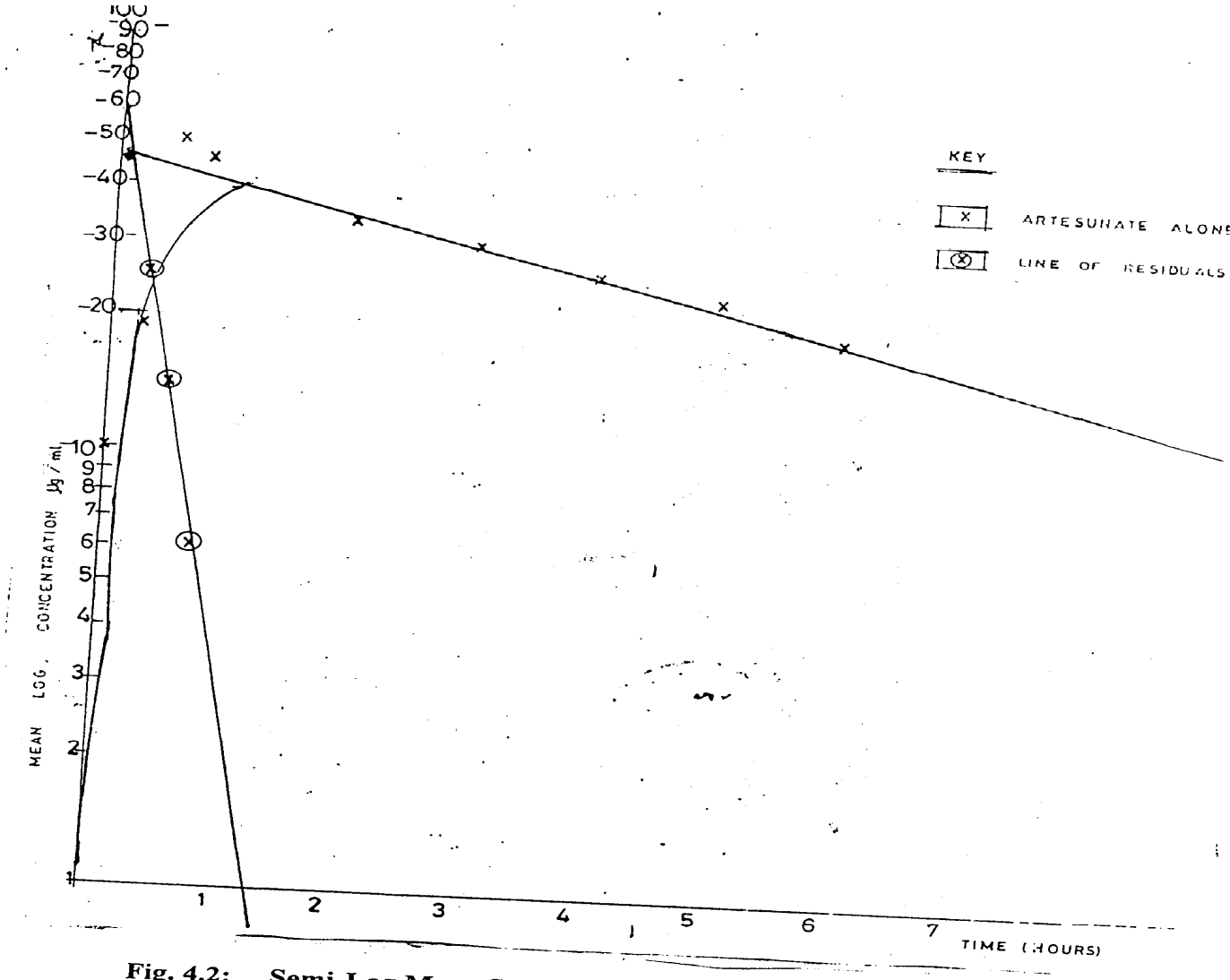

Fig. 4.2: Semi-Log Mean Salivary Concentration versus time graph following oral administration of Artesunate (200mg) to 12 healthy volunteers

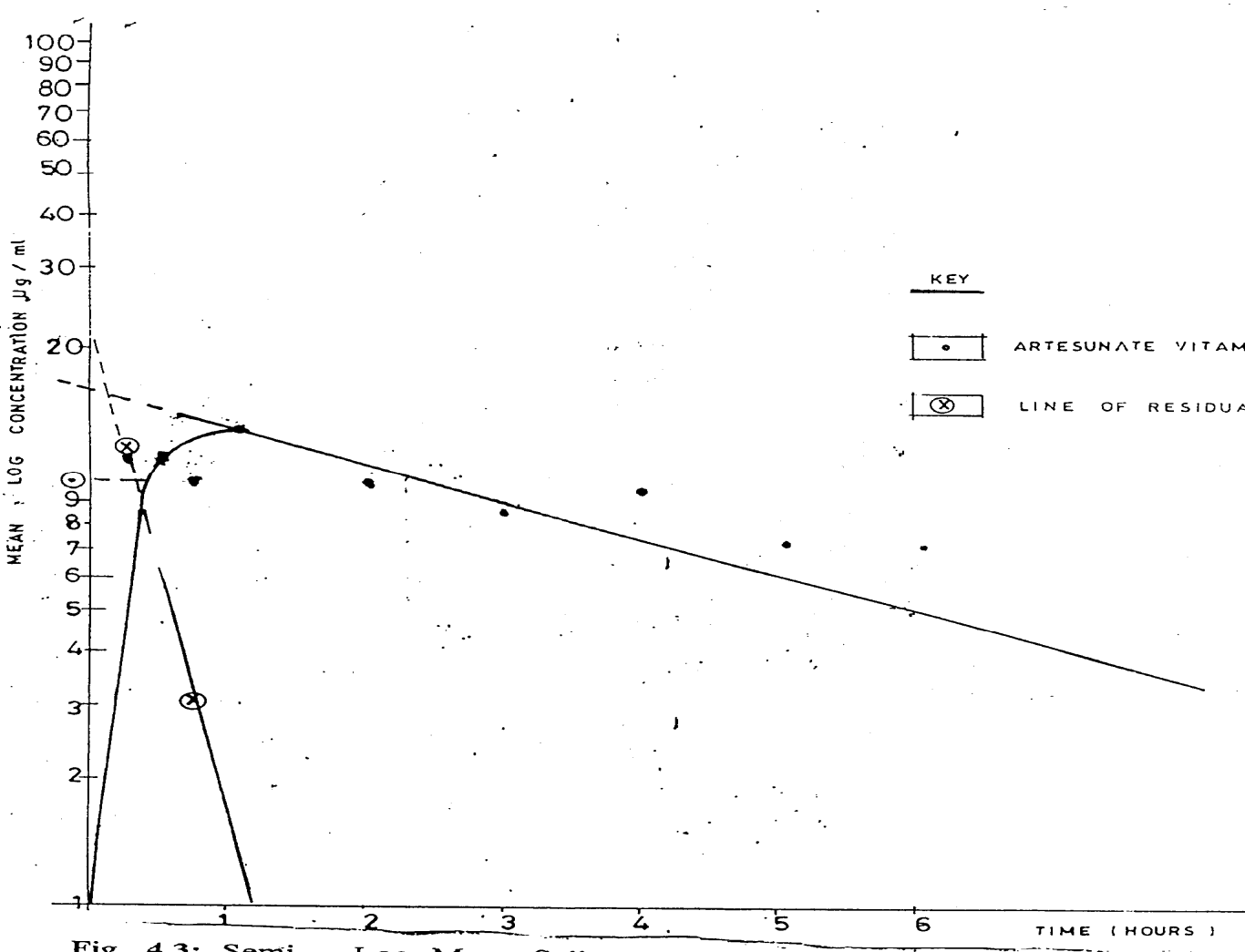

Fig. 4.3: Semi – Log Mean Salivary Concentration versus time graph following oral administration of Artesunate (200mg) + Vitamin C (200mg) to 12 healthy volunteers.

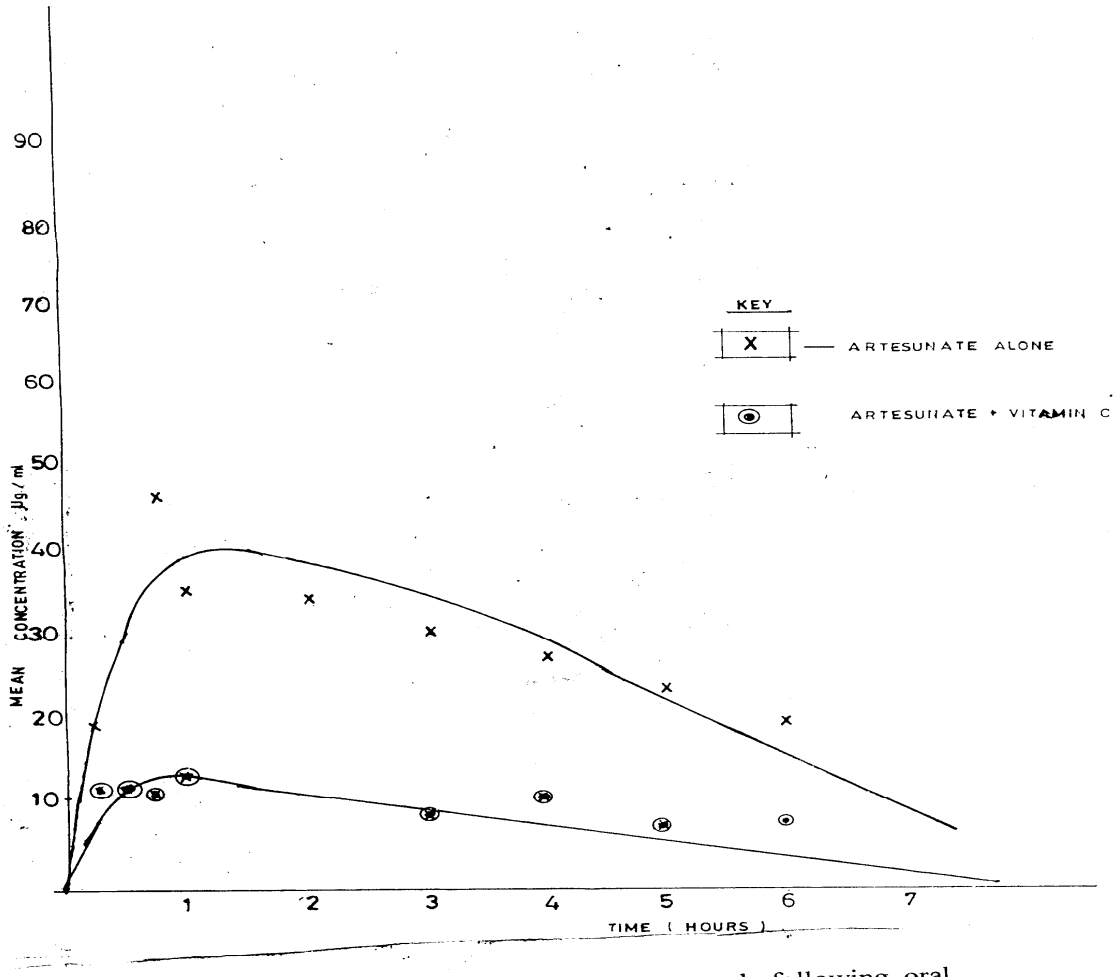

Fig. 4.4: Mean Salivary Concentration versus time graph following oral administration of Artesunate (200mg) alone and concurrent administration of Artesunate (200mg) + Vitamin C (200mg) to 12 healthy volunteers.

Table 4.7 shows the pharmacokinetic parameters of artesunate when administered alone (stage1) and when concurrently administered with vitamin C.

Pharmacokinetic parameters of artesunate when co-administered alone (control) showed the maximum concentration (C_{max}) values to be 40.00±2.83µg/ml and the time to attain the maximum concentration was found to be 1 hour. The lag time was 3.00±0.56 (minutes), elimination half life $t_{1/2}$(el) was 4.50±0.25 (h), absorption half life $t_{1/2}$(ab) to be 0.20±0.04 (h). The value for the absorption rate constant (Ka) was 2.77±0.26 (h^{-1}) and elimination rate constant was 0.15±0.02 (h^{-1}). The total body clearance was 0.10±0.01 (ml/h), volume of distribution (V_d) was 0.69±0.05 (L) and C_O was 46.00±3.78µg/ml.

When artesunate was given concurrently with vitamin C, the pharmacokinetic parameters were given as follows; maximum concentration (C_{max}) was found to be 13.00±1.60µg/ml and time to attain maximum concentration (t_{max}) was 1 hour. The lag time (t_{lag}) was 8.00± 1.60 (minutes) and C_o was ±16.00±2.40µg/ml.The total body clearance (Cl) was 0.47±0.07 (ml/h) and volume of distribution (V_d) was 2.36±0.46 (L). The value of elimination half life $t_{1/2}$(el) was 3.55± 0.53(h) and that of absorption half life $t_{1/2}$(ab) was 0.20± 0.11(h). The area under the curve ($AUC_{0-\infty}$) was found to be 69.44±21.36µg/ml .Absorption rate constant (kab) values was 2.77± 0.78(h^{-1}) and that of elimination rate constant(ke) values was 0.20 ± 0.02.(h^{-1}).

When compared there was statistically significant changes in pharmacokinetic parameters ($P < 0.05$) for C_{max}, $t_{lag,}$ C_o, $AUC_{0-\infty,.}$ Cl. and V_d. However, the changes in t_{max},$t_{1/2ab}$,$t_{1/2el}$ were statistically insignificant.

Table 4.7: Mean Values of Pharmacokinetic Parameters of Artesunate alone and Artesunate +VitaminC (Concurrent Adminstration) and P Values

Pharmacokinetic Parameters	Mean±SEM Artesunate alone	Mean ±SEM Artesunate +VitaminC (concurrent Administration).	P Values
C_{max}(µg/ml	40.00±2.83	13.00±1.60	P<0.05
t_{max}(h)	1.00±1.69	1.00±1.46	P>0.05
Lag Time(mins)	3.00±0.56	8.00±1.60	P<0.05
$t_{1/2ab}$(h)	0.20±0.04	0.20±0.11	p>0.05
$t_{1/2el}$(h)	4.50±0.25	3.55±0.53	p>0.05
$K_{el}(h^{-1})$	0.15±0.02	0.20±0.02	p>0.05
$K_{ab}(h^{-1})$	2.77±0.26	2.77±0.78	p>0.05
C_o(µg/ml)	46.00±3.78	16.00±2.40	p<0.05
Vd(L)	0.69±0.05	2.36±0.46	P<0.05
Cl(ml/h)	0.10±0.01	0.47±0.07	P<0.05
$AUC_{0-\infty}$(µg/ml.h)	238.00±20.78	69.44±21.36	P<0.05

CHAPTER FIVE

5.0.0 DISCUSSION

The pharmacokinetics of artemisinin has been investigated extensively by (Na Bangchang, 1994, De Vries, 1997 and Ashton, 2001). From these studies artemisinin showed inter-individual variability. Inter-individual variability may be as a result of age, weight, dose, environmental factors, genetic influence and diseases such as renal failure.

In this study, artesunate co-administration with ascorbic acid causes decrease in the maximum concentration. The decrease can be attributed to decrease in absorption of artesunate and there are many factors that can influence the extent and the rate of absorption of ingested compounds (Lin et al, 1999). These include physiochemical factors such as molecular size, lipophilicity, solubility, pka and biological factors including gastric and intestinal transit time, lumen pH, membrane permeability and first pass metabolism (Huguchi et al, 1981). The reduction in C_{max} might be as a result of alteration of pH of the gastrointestinal tract and increased first pass metabolism. Artesunate a weakly basic drug must have formed salt with anions present in the Ascorbic acid a weakly acidic drug with the pH of 4.2 and conversely ionize more of the Artesunate at acidic pH. Ions being lipid insoluble do not diffuse and a pH difference across a membrane can cause a differential distribution of weakly acidic and weakly basic drugs. In the previous study on the

use of antimalaria drugs, WHO (2003) found the t_{max} of artemisinin and its derivatives to be between 1-2hrs. Gilbert (2003) in his study of pharmacokinetic parameter of co-artemether when given in combination with quinine found the plasma level of artemisinin derivatives to be lower than when given alone and t_{max} remains constant even after the combined therapy.

There was an increase in lag time for the test drug and this increase might be due to alteration of pH of the gastrointestinal tract since both drugs are absorbed in the intestine.

The area under the concentration time-curve decreased for the test and this indicated that ascorbic acid must have increased the activity of microsomal drug metabolizing enzyme (cytochrome p-450) through glycogenesis pathway (Food and Nutrition Board, Institute of medicine, 2000) which in turn enhanced the activity of the enzyme responsible for the metabolism of artesunate thereby increasing rate of metabolism. In the study of effect of food on the pharmacokinetics of artemisinin, it was found that hepatic metabolism is the main route of elimination (Dien et al, 1997).

In support of the reasons given above Niu et al, (1985) suggested that hepatic metabolism is the route of elimination of artemisinin. In comparison with other tissues, artesunate disappearance was more rapid when incubated with rat liver slice indicating the liver to be the major site of metabolism. Also disappearance of

the structural analogue artesunate was NADPH- dependent when incubated with rat liver microsomes indicating the cytochrome P-450 enzyme to be involved in its metabolism (Leskovac and Theohatrides, 1999).

Wilkinson and Shand, (1975) also found AUC to be governed by intrinsic hepatic clearance and extensive liver extraction. There was increase in the clearance and this also affected the AUC. The increase in the clearance resulted to decrease in the AUC.

The change in volume of distribution V_d was statistically significant ($p<0.05$). The increased values are expected since area under the curve was generally low compared to the control groups. The apparent volume of distribution is inversely proportional to the area under the curve.

For the first order rate process the total body clearance is the product of kel and V_d (Levine, 1970). Thus, the increase in the total body clearance might be due to a corresponding increase in the apparent volume of distribution (V_d).

The pharmacokinetic and tolerability of artesunate and amodiaquine alone and in combination in 15 healthy volunteers were carried out and it was observed that the mean AUC was lower for dihydroartemisinin (ratio 67%, 95%Cl,51-88%) and desethylamodiaquine (ratio 65%, 95%, Cl 46-90% , p=0.0015) when compared with monotherapy. The total drug exposures to both drugs were reduced significantly (Orrell et al, 2008).

In the study of interaction between artemether-lumenfantrine and quinine in healthy subjects, the plasma level of artemether and dihydroartemisinin appeared to be lower when co-artemether was given in combination with quinine compared to co-artemether alone. The t_{max} and $t_{1/2}$ for artemether and dihyroartemisinin was comparable following both treatments. There was no change in t_{max} and $t_{1/2.}$ (Gibert, 2003).

Similarly, clinical pharmacokinetics of artemether-lumenfantrin, including the effect of food or the influence of food intake on the bioavailability of co-artemether was carried out. The result obtained was that oral bioavailability (F) of artemether was increased more than two fold and that of lumenfantrin around 16 fold after fat meal compared with fasting. Dihydroartemisinin exposure increased in the same proportion as for artemether. The mean apparent plasma $t_{1/2\beta}$ of lumenfantrin was doubled from 35.1 (fasted) to 71.3 hours under fed conditions. The increase in systemic exposure in the presence of food was statistically significant for all three compounds (Gilbert, 2003).

In multiple dose studies with artemisinin in both healthy subjects and patients, peak plasma artemisinin concentrations on the 6^{th} day were reduced to 20% of those on day one. There was no change in $t_{1/2.}$ (Michiel, et al, 1999). It was suggested that auto –induction of enzymes in the liver or gut were responsible for

this time-dependent increase in first-pass effect (Michiel, et al, 1999). Time dependent pharmacokinetics has also been observed in studies with artemether. Interaction with grape fruit juice, a strong inhibitor of CYP-3A4 in gut mucosa cell, showed more than a two fold increase in artemether bioavailability which suggests a role of CYP3A4 in the first pass elimination of artemether (Michiel, et al, 1999).

Similarly, in the study of effect of food on the pharmacokinetic parameters of artemisinin, it was found that there was no significant change in plasma clearance, bioavailability, maximum concentration, time to attain maximum concentration, area under the curve after administration of food (Dien, et al 1997) while a decrease in maximum concentration during a multidose regimen was found recently without a difference in elimination rate (Hassan et al, 1999).

In an interaction study between artesunate and mefloquine there was no significant impact on the absorption and distribution of mefloquine but artesunate decreases the bioavailability of mefloquine (Karbwang et al, 1994) while in another study of pharmacokinetic and pharmacodynamic evaluation of two artemisinin-mefloquine combinations in adult falciparium malaria, the AUC_S after the first dose were two fold higher than when combined with mefloquine. It was concluded that artemisinin does not induce or influence mefloquine metabolism at clinical doses, Whereas mefloquine may have inhibited the metabolism of artemisinin (Svensson et al, 1998). There was rapid metabolism and low bioavailability in rodents and

human when curcumin was used in combination with α, β-arteether in the treatment of malaria (Dalavaikodihalli et al, 2006).

Investigation was carried out to determine whether co-administration of the artesunate and artemisinin alters the clearance of either drug and it was found that Artesunate did not alter elimination of artemisinin. However, dihydroartemiusinin elimination was inhibited by artemisinin (Zhang et al, 2001).

The Synergistic effect was observed in-vitro with artemisone and mefloquine and in-vivo reports were similar. The combination of artemisone and chloroquine with clindamycin had a synergistic effect in-vivo, particularly with chloroquine against the chloroquine resistant *P.yoeli* and less apparent with clindamycin (Nagamune et al, 2007).

CHAPTER SIX

6.0.0 CONCLUSIONS AND RECOMMENDATIONS.

6.1.0 CONCLUSIONS

Co-administration of 200mg of artesunate and 200mg of ascorbic acid has led to a decrease in the maximum salivary concentration of artesunate (C_{max}) and area under curve from zero to infinity ($AUC_{0-\infty}$) in healthy male volunteers and the decrease was statistically significant ($p<0.05$) when compared to the control. On the contrary, the Lag time (t_{lag}), plasma clearance (Cl) and volume of distribution (V_d) increased significantly ($p<0.05$) from values obtained in the control group. Other parameters like elimination half life ($t_{1/2el}$), absorption half life ($t_{1/2ab}$), elimination and absorption rate constant (k_e,k_{ab}) respectively recorded insignificant changes($p>0.05$). The time to attain maximum concentration (t_{max}) remained constant. The results obtained from the study all fitted into first order kinetics.

6.2.0 RECOMMENDATIONS

The concurrent administration of Artesunate and Ascorbic acid should be discouraged because the plasma concentration of Artesunate is low when co-administered with Ascorbic acid which can lead to reduced therapeutic effect. If prescribed together, it is advisable for Artesunate tablets to be taken first and thereafter Ascorbic acid can be given so as to achieve maximum therapeutic effect. We should also discourage taking dietary supplement of ascorbic acid along with Artesunate tablet.

Despite the efforts made to study the pharmacokinetic interaction of Artesunate and Ascorbic acid, further research is required to overcome the limitations of this work which are lack of sensitive equipments such as HPLC for analysis and improper compliance of volunteers to the protocols of the study. It is therefore recommended that further study on the delayed administration of Ascorbic acid after Artesunate should be carried out to see the effect on the pharmacokinetic parameters of Artesunate.

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APPENDICES

APPENDIX I

The Ages and Weights of Twelve Volunteers that Ingested 200mg of Artesunate Tablets and 200mg of Ascorbic Acid Tablets

Volunteers	Sex	Age (yrs)	Weight (kg)
1	Male	28	60
2	“	30	70
3	“	26	65
4	“	27	64
5	“	23	60
6	“	24	62
7	“	31	72
8	“	29	69
9	“	32	70
10	“	24	66
11	“	23	65
12	“	25	60
Mean ± SEM		26.83 ± 0.91	65.25 ± 1.22

APPENDIX II

Concentration values of different volunteers n=12 of Biological samples (Saliva) obtained from administration of Artesunate alone (µg/ml)

Time (min)	VL1	VL2	VL3	VL4	VL5	VL6	VL7	VL8	VL9	VL10	VL11	VL12
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15.00	15.00	20.40	22.00	16.20	18,20	26.40	15.57	23.00	20.00	17.20	18.20	15.00
30.00	36.00	58.00	64.00	54.00	56.00	45.00	37.10	50.80	38.00	54.00	45.00	58.00
45.00	30.40	42.00	60.00	66.00	53.00	43.00	30.50	42.80	40.00	50.00	37.00	52.00
60.00	28.80	32.40	29.50	64.00	51.20	32.00	28.00	32.00	35.00	43.00	32.00	40.20
120.00	27.40	28.80	28.80	54.40	50.00	27.00	27.20	30.40	32.00	38.00	26.00	34.20
180.00	27.00	28.00	25.20	58.00	35.20	25.00	24.00	27.00	23.00	36.00	24.50	30.50
240.00	26.40	27.20	20.40	52.80	27.20	23.00	20.00	24.00	21.00	30.00	21.00	24.00
300.00	22.00	20.50	18.00	45.00	25.20	22.00	19.00	22.00	20.00	25.00	20.00	22.00
360.00	19.20	17.20	16.50	30.20	21.00	18.00	14.00	16.00	19.00	21.00	17.00	15.00

APPENDIX III

Concentration values of different volunteers n=12 of Biological samples (Saliva) obtained from administration of Artesunate and Ascorbic acid (µg/ml)

Time (min)	VL1	VL2	VL3	VL4	VL5	VL6	VL7	VL8	VL9	VL10	VL11	VL12
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15.00	12.80	15.20	4.00	6.00	21.20	8.50	20.80	4.80	7.80	11.00	13.20	9.30
30.00	22.00	14.80	6.00	2.20	14.60	7.20	15.80	6.20	2.20	21.00	11.00	10.00
45.00	11.20	10.00	6.80	3.20	18.00	5.20	18.00	6.80	3.20	11.00	11.00	13.00
60.00	22.00	15.80	2.40	9.50	24.00	4.80	24.00	12.00	10.40	22.00	12.00	7.50
120.00	8.40	14.80	10.20	2.20	25.20	5.80	25.20	10.00	2.20	8.20	10.40	10.20
180.00	4.00	13.20	2.20	2.30	12.00	2.40	12.00	8.40	2.30	4.00	19.00	11.20
240.00	5.40	11.80	10.00	4.00	18.20	3.50	19.20	6.20	4.00	7.80	12.40	9.40
300.00	2.20	10.00	4.80	5.20	9.50	4.80	9.50	4.80	5.20	5.20	7.20	5.00
360.00	4.80	6.00	4.80	4.00	10.40	2.00	10.40	2.80	4.20	5.20	3.20	4.20

APPENDIX IV

Individual Pharmacokinetic Parameters for Artesunate and vitamin C (Concurrent Administration).

parameters	VL_1	VL_2	VL_3	VL_4	VL_5	VL_6	VL_7	VL_8	VL_9	VL_{10}	VL_{11}	VL_{12}
C_{max}[µg/ml]	22.00	19.00	10.00	10.00	25.00	9.00	25.00	12.00	10.00	12.00	19.00	13.00
$T._{max}$ (h)	1.00	3.00	2.00	1.25	2.00	0.45	2.00	2.00	1.00	1.00	3.00	0.45
Lag Time mins)	5.00	7.50	5.00	15.00	15.00	15.00	5.00	1.00	7.50	1.00	1.00	3.00
$t_{½\ ab}$ (h)	1.36	0.23	0.43	0.65	0.34	0.27	0.19	0.38	0.16	0.18	0.34	0.30
$t_{½el}$ (h)	2.0	8.88	3.98	3.64	4.25	2.44	5.33	2.96	4.24	3.39	3.85	2.26
Ke[h^{-1}]	0.51	2.99	1.61	10.82	2.04	2.59	3.67	1.84	1.13	3.89	2.06	2.37
Ke[h^{-1}]	0.34	0.08	0.17	0.19	0.61	0.28	0.13	0.24	0.16	0.20	0.18	0.31
Co[µg/ml]	20.00	25.00	13.00	12.00	35.00	10.50	32.00	17.00	10.50	32.00	25.00	23.00
Vd (L)	3.25	0.71	2.90	4.51	1.27	5.98	1.11	3.34	4.00	2.58	1.35	3.17
Cl (ml/h)	1.09	0.10	0.50	0.90	0.21	1.69	0.15	0.78	0.66	0.52	0.24	0.97
$AUC_{o-\infty}$[µg/ml.h]	61.44	280.75	62.75	44.08	157.28	33.44	179.75	59.85	49.93	77.6	148.23	63.03

APPENDIX V

Individual Pharmacokinetic Parameters of Artesunate Alone

Pharmaco-kinetic Parameters	VL_1	VL_2	VL_3	VL_4	VL_5	VL_6	VL_7	VL_8	VL_9	VL_{10}
(C_{max} [µg/ml]	36.00	58.00	64.00	66.00	56.00	45.00	37.00	52.00	40.00	54.00
$T._{max}$ (h)	0.30	0.30	0.30	0.45	0.30	0.30	0.30	0.30	0.45	0.30
Lag Time (mins)	3.00	5 .00	3.0	3.00	7.50	3.00	1.00	3.00	7.50	3.00
$t_{1/2\ ab}$ (h)	0.38	0.38	0.43	0.26	0.26	0.25	0.25	0.33	0.10	0.28
$t_{½\ el}$ (h)	4.38	3.88	2.00	3.50	3.25	3.00	3.75	3.50	5.38	3.85
Kab (h^{-1})	2.10	2.38	1.60	2.69	2.84	2.77	2.77	4.15	2.92	0.49
Ke (h^{-1})	0.19	0.24	0.35	0.20	0.17	0.23	0.18	0.20	0.13	0.16
Co [µg/ml]	48.00	72.00	76.00	80.00	78.00	56.00	42.00	58.00	46.00	64.00
Vd (L)	0.80	0.80	0.98	0.44	0.59	0.86	0.98	0.85	0.66	0.64
Cl (ml/h)	0.15	0.20	0.34	0.08	0.10	0.20	0.18	0.17	0.83	0.15
$AUC_{o-\infty}$[µg/ml.h]	248.63	244.33	205.03	450.51	338.39	232.42	203.53	235.20	301.87	313.29

MAY, 2009

EFFECTS OF ASCORBIC ACID ON THE PHARMACOKINETICS OF ARTESUNATE IN HEALTHY MALE VOLUNTEERS

BY

OLOGUNLA, FOLASHADE VICTORIA
(M.Sc/Pharm.Sci/41888/04-05)

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, AHMADU BELLO UNIVERSITY ZARIA, IN PARTIAL FULFILMENT FOR THE AWARD OF DEGREE OF MASTER OF SCIENCE PHARMACOLOGY

DEPARTMENT OF PHARMACOLOGY AND CLINICAL PHARMACY, FACULTY OF PHARMACEUTICAL SCIENCES, AHMADU BELLO UNIVERSITY ZARIA, NIGERIA

MAY, 2009

DECLARATION

I hereby declare that the work reported in this thesis was carried out by me under the supervision of Professor Ibrahim Abdu-Aguye and Dr, Joseph Anuka. It has not been presented in any previous application for higher degree. The work of other investigators are acknowledged and referenced accordingly.

---------- ----------

Ologunla, Folashade Victoria **Date**

CERTIFICATION

This thesis entitled effects of ascorbic acid on the pharmacokinetic parameters of artesunate in healthy male volunteers by Ologunla, Folashade Victoria meet the regulations governing the award of the degree of Master of Science of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentations.

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Prof. I. Abdu-Aguye Date
Chairman, Supervisory Committee

---------- ----------

Dr. J. A Anuka Date
Member, Supervisory Committee

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Dr. (**Mrs.) B. B Maiha**
Head of Department Date

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Prof. S. A Nkom Date

Dean, Postgraduate School

DEDICATION

I hereby dedicate this work to God Almighty who has does carry us far.

ACKNOWLEDGEMENT

I am most grateful to the Almighty God for His guidance and protection throughout the period of this work. I also wish to thank my supervisors Prof. Ibrahim Abdu-Aguye and Dr. Joseph Anuka for their guidance and corrections and above all for finding time to work with me throughout the period of this work.

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ABSTRACT

Twelve healthy young adult male volunteers with average age of 26± 4 years and average weight of 66.25 ± 5kg took part in this study. The study was designed with oral dose in two stages. In stage one; artesunate (200mg) was given alone and after a wash out period of two weeks stage two commenced with co-administration of artesunate (200mg) and ascorbic acid (200mg). Saliva sample were collected for analysis at 0, 15, 30, 45mins, 1, 2, 3, 4, 5, 6hrs in both stages. A reliable spectrophotometer was used for saliva concentration determination. The pharmacokinetic parameters were generated from saliva concentration of artesunate using suitable formulae. The maximum concentration and area under curve from 0 - ∞ decreased significantly ($p < 0.05$) when compared to the control. The lag time and plasma clearance increased significantly ($p < 0.05$) from values obtained from the control group. There were insignificant increases in elimination half life, absorption half life, elimination and absorption rate constants, volume of distribution and time to attain maximum concentration. In conclusion, it was observed that ascorbic acid co-administration with artesunate altered the pharmacokinetic parameters of artesunate. The result implies that ascorbic acid reduced the amount of artesunate

available to the body tissues for utilization such concurrent administration should therefore be discouraged.

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ABBREVIATIONS

Abbreviations	Meaning
B.P	British Pharmacopoeia
^{o}C	Degree Centigrade (Celsius)
g	Gram
µg	Microgram
mg	Milligram
ml	Millilitre
min	Minute
hr	Hour
HCl	Hydrochloric acid
NaoH	Sodium hydroxide
u.v	Ultraviolet
nm	Nanometer
kg	Kilogram
L	Litre
%	Percentage

rpm	Rotation per minute

LIST OF APPENDICES