OBAFEMI AWOLOWO UNIVERSITY

PHARMACOKINETIC INTERACTIONS BETWEEN QUININE AND CIPROFLOXACIN IN HEALTHY VOLUNTEERS

BY

ADEGBOLA, ADEBANJO JONATHAN

B.PHARM. (IFE)

PHP10/11/H/0528

A THESIS SUBMITTED

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE

(PHARMACEUTICAL CHEMISTRY)

IN THE

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

FACULTY OF PHARMACY

OBAFEMI AWOLOWO UNIVERSITY, ILE-IFE

2014.

OBAFEMI AWOLOWO UNIVERSITY, ILE IFE, NIGERIA

HEZEKIAL OLUWASANMI LIBRARY

POSTGRADUATE THESIS

AUTHORISATION TO COPY

AUTHOR: Adegbola Adebanjo Jonathan

TITLE: Pharmacokinetic Interactions Between Quinine and Ciprofloxacin in Healthy Volunteers.

DEGREE: M.Sc. (Pharmaceutical Chemistry)

YEAR: 2014

I, Adegbola Adebanjo Jonathan, hereby authorize the Hezekial Oluwasanmi library to copy my thesis, in whole or in part, in response to requests from individual researchers or organisations for the purpose of private study or research.

....

Signature **Date**

CERTIFICATION

This is to certify that the thesis work titled 'Pharmacokinetic Interaction between Quinine and Ciprofloxacin in Healthy Volunteers was carried out by, Adegbola Adebanjo Jonathan for the award of a M.Sc. degree in the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.

..... **.....**
Dr. Soyinka Julius O. **Date.**
(Supervisor)

..... **.....**
Dr. Idowu Thomas O. **Date**
(Head of Department)

DEDICATION

This work is dedicated to God Almighty. Also, to Oluwademilade-Ayo Hallelujah, a queen given to us by God almighty during the course of this work.

ACKNOWLEDGEMENT

I express my profound gratitude to almighty God who has made it possible for me to end this work in the fruition. For every meaningful project, God is always there during its conception and execution. Thus, I am grateful to Him for He did not leave me alone.

Also, I am greatly indebted to my supervisor, Dr. J.O. Soyinka for a pivotal role he played during the conception and birth of this work. I appreciate your support and thoroughness in taking me through the work. Despite his stretched schedule, he created time for me to ensure that this work amount to something.

I owe a huge appreciation to Prof. O.O. Bolaji for the support I received from him during the course of this work. I am delighted to express that I have benefited without measure from the eminent role you play in developing researches focus on drug metabolisms and pharmacogenetics.

Similarly, I cannot but express my gratitude to my sweetheart, Mrs. I.A. Adegbola, for her supports and encouragements which served as launch pad for me during this work. I must also appreciate my kids, Praise, Glory and Grace, for their understanding. I pray that we shall reap the harvest of this work bountifully.

My appreciation goes to my teachers Prof. O.A. Ogundaini, Prof. T. A. Olugbade, Prof. C.O. Onyeji, Dr. T.O. Idowu and Dr. B.J. Taiwo. You have all impacted and sharpened my life in tremendous ways, in particular, intellectually. I appreciate the efforts of other members of staff of the Department of Pharmaceutical Chemistry who contributed in one way or the other towards the success of this work.

Finally, I thank Mr. B.A. Adeagbo for the guidance, contributions and supports I received from him.

TABLE OF CONTENTS

		Pages
Title page		i
Authorisation to copy		ii
Certification		iii
Dedication		iv
Acknowledgement		v
Table of contents		vi
List of Tables		x
List of Figures		xii
Abstract		xiii
CHAPTER ONE		
1.0	Introduction	1
1.1	The Burden of Malaria in our Society	1
1.2	Quinine Discovery and Overview of Quinine in the Treatment of Malaria	5
1.3	Chemistry of Quinine	11
1.3.1	Physicochemical properties of Quinine	12
1.3.2	Mechanism of Action of Quinine	14

1.3.3 Potential Explanations for Quinine Treatment Failure 14

1.4 Pharmacokinetic Principle 17

1.4.1 Rates of Reaction 18

1.4.2 Pharmacokinetic Models 20

1.4.3 Pharmacokinetic Parameters 22

1.4.4 Pharmacokinetic Applications 23

1.4.5 Drug Metabolism 27

1.4.6 Cytochrome P450 28

1.5 Pharmacokinetic Properties of Quinine 32

1.5.1 Absorption and Bioavailability of Quinine 32

1.5.2 Distribution, Metabolism and Elimination of Quinine 33

1.6 Present State of Knowledge on Quinine interactions with Other Drugs 37

1.6.1 Co-morbidity of Malaria with Bacterial Infections 40

1.7 Properties and Actions of Ciprofloxacin 41

1.8 HPLC Analytical Methods for the Analysis of Quinine and its Metabolite 44

1.9 Objectives and Rationales for the study 46

1.9.1 The specific objectives of this research 46

1.9.2 The rationale for the study 46

CHAPTER TWO 47

2.0 Experimental and Methodology 47

2.1 Sample collection 47

2.1.1 Ethical Clearance for the Protocol 47

2.1.2 Recruitment of Healthy Volunteers 47

2.1.3 Inclusion and Exclusion Criteria 48

2.1.4 Administration of Drugs and Collection of Blood Samples 48

2.2 Experimental Study 49

2.2.1 Chemicals and Reagents 49

2.2.2 Chromatographic Condition and Instrumentation 49

2.2.3 Preparation of Standard Solutions and Solvent System 49

2.2.4 Analytical Procedures 50

2.3 Validation of Analytical Methods 51

2.3.1 Calibration Curves 51

2.3.2 Intra and Inter-day Precision and Accuracy 51

2.3.3 Recovery 51

2.4 Pharmacokinetic and Statistical Analysis 52

CHAPTER THREE 53

3.0 Results 53

3.1 Chromatograms of extract of the blank plasma containing the analytes 53

3.2 Calibration curve for quinine and 3-hydroxyquinine in plasma 58

3.3 Precision and accuracy of the analytical method 60

3.4 Plasma levels of quinine and its metabolite 64

3.5 Derived pharmacokinetic parameters for quinine and 3-hydroxyquinine 65

3.6 Summary of pharmacokinetic parameter of quinine and 3-hydroxyquinine 74

CHAPTER FOUR 77

4.0 Discussion and Conclusion 77

4.1 Analytical methods 77

4.2 Co-administration of quinine and ciprofloxacin 80

4.3 Pharmacokinetic profiles of quinine and 3-hydroxyquinine 80

4.4 Inhibition of metabolism of quinine when co-administered with Ciprofloxacin 83

4.5 Conclusion 85

REFERENCES 87

APPENDICES 101

LIST OF TABLES

	Tables	page
1.1	Some substrates, inducers and inhibitors of the major CYP450 enzymes	31
3.1	Result of Precision Studies	61
3.2	Result of Recovery in Plasma	62
3.3	Accuracy of Analytical Method	63
3.4	Some derived Pharmacokinetic parameters of quinine following single oral Administration of 600 mg dose of quinine sulphate to each twelve healthy Volunteers.	68
3.5	Some derived Pharmacokinetic parameters of quinine following administration of 600 mg single oral dose of quinine sulphate taking concurrently with multiple Dose of 500 mg twice of Ciprofloxacin to each twelve healthy volunteers.	69
3.6	Some derived Pharmacokinetic parameters of 3-hydroxyquinine following Single oral administration of 600 mg dose of quinine sulphate to each twelve Healthy volunteers.	71
3.7	Some derived Pharmacokinetic parameters of 3-hydroxyquinine following concurrent Administration of multiple doses of Ciprofloxacin and Single oral dose of 600 mg Quinine sulphate to each of twelve healthy volunteers.	72
3.8	Comparision of the ratio of AUC metabolite to that of unchanged drug for Quinine alone with that of quinine plus ciprofloxacin (metabolic ratio).	73
3.9	Summary of pharmacokinetic parameters of quinine without and with Concurrent administration of ciprofloxacin.	75

3.10 Summary of Pharmacokinetic parameters of 3-hydroxyquinine following Administration of 600 mg quinine sulphate with or without concurrent administration of ciprofloxacin. 76

LIST OF FIGURES

Figures		pages
1.1	Chemical Structure of Ciprofloxacin, Pyrimethamine, Quinine, Quinine salt and its metabolite	13
1.2	Metabolic Biotransformation of Quinine	36
3.1	Chromatogram of blank plasma without analytes showing endogenous compound	54
3.2	A chromatogram of a blank plasma spiked with Ciprofloxacin, Internal Standard Quninine and 3-Hydroxyquinine	55
3.3	Chromatograms obtained from the extract of test plasma sample of a volunteer for plasma sample 2 hours after administration of single oral dose of 600 mg quinine alone	56
3.4	Chromatograms obtained from the extract of test plasma sample of a volunteer for plasma sample 2 hours after co-administration of 600 mg single oral dose quinine with ciprofloxacin.	57
3.5	Calibration curves for quinine and 3-hydroxyquinine in plasma	59
3.6	Mean plasma concentration versus time profiles for quinine following Single oral administration of 600 mg of quinine sulphate with and without Multiple doses of ciprofloxacin.	65
3.7	Mean plasma concentration versus time profiles for 3-hydroxyquinine Following single oral administration of 600 mg of quinine sulphate with and Without multiple doses of ciprofloxacin.	66
4.1	Quantitative difference in the derived pharmacokinetic parameters of quinine when it was administered alone and when administered with ciprofloxacin	82

ABSTRACT

This study determined the baseline pharmacokinetic parameters of quinine and its major metabolite (3-hydroxyquinine) and evaluated the effect of concurrent administration of ciprofloxacin on the pharmacokinetic parameters of quinine in healthy volunteers with a view of obtaining information that will guide the usage of quinine when co-administered with ciprofloxacin.

Ethical approval was obtained from the Ethics Committee of the Institute of Public Health, Obafemi Awolowo University, Ile Ife and written informed consent was obtained from the healthy volunteers. Each subject was assessed at screening to be healthy by physical examination, medical history and routine laboratory evaluations. The study was implemented as a 2-period design. In period 1, a single oral dose (600 mg) of quinine sulphate was given to each of twelve volunteers and blood samples were withdrawn at pre-determined intervals over 48 hours. After a wash out period of 1 month, each volunteer received multiple oral doses of 500 mg ciprofloxacin tablet every 12 hours for 7 days and a single oral dose of 600 mg quinine sulphate was then given concurrently with the 11^{th} dose of ciprofloxacin, and blood samples were taken at predetermined intervals again over 48 hours after drug administration. The blood samples were centrifuged to obtain plasma which was stored in the freezer at -20 °C until the samples were analysed for plasma levels of quinine and its main metabolite, 3-hydroxyquinine, by High Performance Liquid Chromatographic method. The pharmacokinetic parameters of quinine with or without ciprofloxacin were determined and the differences between the two pairs of data were evaluated by the Student's t-test. A p-value of < 0.05 was considered statistically significant.

Concurrent administration of quinine and ciprofloxacin resulted in significant ($p < 0.05$) increased elimination half life ($t_{1/2}$), maximum plasma concentration (C_{max}) and area under the curve ($AUC_{0-\infty}$) of quinine by 32 %, 19 % and 49 % respectively. Similarly, ciprofloxacin caused a marked decrease of 31 % in the plasma clearance (Cl_p) of quinine. The C_{max} and $AUC_{0-\infty}$ of 3-hydroxyquinine were also significantly reduced when ciprofloxacin was co-administered with quinine by 53 % and 44 % respectively. In addition, the metabolic ratio of quinine was markedly decreased by 63 % when co-administered with ciprofloxacin. These results showed that the formation of 3-hydroxyquinine had been hindered in the presence of ciprofloxacin. This might be attributed to the inhibition of an enzyme, CYP 3A4, which is responsible for the conversion of quinine to 3-hydroxyquinine.

The study concluded that there is a modest and significant pharmacokinetic (metabolic) interaction between quinine and ciprofloxacin *in vivo* when co-administered.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

1.1 The Burden of Malaria in Our Society

Malaria is an important cause of death and illness in children and adults, especially in tropical countries. Malaria control requires an integrate approach, including prevention (primarily vector control) and prompt treatment with effective antimalarials. It is said to be the most important parasitic disease that inflicts humans today (WHO malaria treatment guidelines, 2010). It is one of the most serious complex and refractory health problem facing humanity this century and is by far the most important tropical disease causing great suffering and loss of life worldwide (Weil, 2011). Malaria is a leading killer of children under five and it kills a child every minute in Africa (UNICEF, 2013). In 2012 alone, 207 million malaria cases leading to approximately 627,000 malaria deaths mostly among African children were reported (WHO, 2013). This report further revealed that 40 % of world population (about 3.4 billion people) were at risk of malaria, with populations living in sub-Saharan Africa having the highest risk of acquiring malaria. Survey globally also showed that 80 % of malaria deaths occur in just 14 African countries. Together, the Democratic Republic of Congo and Nigeria account for over 40 % of the estimated total of malaria deaths globally. Malaria remains the foremost killer disease in Nigeria where it accounts for over 25 % of under-5 mortality, 30 % childhood mortality and 11 % maternal mortality. It also account for about 60% of out-patient visits and 30 % of all hospital admissions

and it consistently ranks among the three most common causes of death in Nigeria with 50 % of the population experiencing at least one episode of malaria each year (FMOH, 2007).

Malaria is caused by five species of parasites of the genus *Plasmodium* that affect humans (*P. falciparum, P. vivax, P. ovale, P. malariae and P. knowlesi*. Malaria due to *P. falciparum* is the most deadly form and it predominates in Africa. *P. vivax* is less dangerous but more widespread, and the other three species are found much less frequently (WHO, 2013). Malaria parasites are transmitted to humans by the bite of infected female mosquitoes of more than 30 anopheles mosquito species (WHO, 2013). However, congenital malaria and acquisition through infected blood transfusion are well described (Kitchen *et al., 2006*, Falade *et al.,* 2007). Mixed infections caused by more than one Plasmodium species are frequent but under recognised (Crawley *et al.,* 2010). Around 90% of all malarial cases were caused by *P. falciparum* and it is responsible for most malaria-related deaths worldwide. Also it is the predominant Plasmodium species in sub-Saharan Africa where transmission intensity and population at risk vary considerably between and within countries (Guerra *et al.,* 2008). Almost all populations at medium and high levels of risk live in sub-Saharan Africa, where the burden of disease, death, and disability from falciparum malaria is high (Gething *et al.,* 2010).

In Asia, *P. vivax* is now emerging as the dominant Plasmodium species and it is the most prevalent of the five human malaria parasites outside Africa (Price *et al.,* 2009). It is mostly absent from Central and West Africa because a high proportion of the population have the Duffy-negative phenotype, which prevents erythrocyte invasion by the parasite. In other tropical regions of the world, *P. vivax* coexists with other Plasmodium species and mixed infections are common (Crawley *et al.,* 2010). The transmission rates are low in most regions where *P. vivax* is prevalent therefore affected populations do not achieve high levels of immunity to this parasite

and people of all ages are at risk of infection, although children are more often ill (Crawley *et al.,* 2010).There is increasing evidence that *P. vivax* is responsible for substantial morbidity and mortality, especially in infants (Poespoprodjo *et al.,* 2009). Control is not straightforward because of the difficulty of achieving radical cure by elimination of dormant liver stages (hypnozoites). Infection with *P. malariae* occurs in most malaria-endemic areas, but is much less common than is infection with *P. falciparum or P. vivax*. *P. ovale* is rare outside Africa. *P. knowlesi*, a zoonosis found throughout Southeast Asia, is often misidentified as *P. malariae*, although the clinical course is more severe and fatalities have been described (Cox-Singh *et al.,* 2008).

Several African countries that have achieved high coverage with insecticide-treated nets, indoor residual spraying, and effective treatment programmes have reported a pronounced decline in malaria burden, accompanied, in some instances, by a sharp fall in all-case mortality in children younger than 5 years of age (KleinSchmidt *et al.,* 2009). Enhanced optimism and a marked increase in funding for malaria control have prompted recent calls to revisit the possibility of malaria elimination in some countries and regions. The severity and course of a clinical attack depend on the species and strain of the infecting plasmodium parasite, as well as the age, genetic constitution, malaria-specific immunity, and nutritional status (Caulfield *et al.,* 2004).

There are concerns about some complications associated with malaria especially in children. Untreated malaria in a young child or in a non-immune individual may become complicated. The patient may present with very high body temperature, drowsiness, convulsions and coma indicating heavy parasitaemia, impaired consciousness (prostration or coma), seizures, respiratory distress, metabolic acidosis, severe anaemia, hypoglycaemia and cerebral malaria (Crawley *et al.,* 2010).

Cerebral malaria is defined by WHO as unrousable coma in a patient with *P. falciparum* (or other species) parasitaemia in whom other causes of encephalopathy have been excluded (WHO, 2013). Although the term implies a distinct disease entity, the clinical syndrome is highly variable. Antimalarial drugs have formed the mainstay of treatment. The recommended treatment of severe complicated malaria is intravenous quinine or artemisinin derivatives (Crawley *et al.,* 2010). Intravenous infusion of quinine should be given slowly over 8 hours to avoid cardiac complications. This should be followed by oral quinine tablets for a total of 7 days once the patient is conscious and can take drug orally. Thus, over the year, intravenous quinine remains standard treatment for patients with cerebral or severe malaria and still remains the first line drug in most African countries (Achan *et al.,* 2011).

A concern at present is the emergence and rapid spread multi-drug resistance *P. falciparum*. Antimalarial drug resistance poses a major threat to malaria control efforts. Drug resistance is the degree to which a disease or disease-causing organism remains unaffected by a drug which was previously able to eliminate it. In the case of malaria, it is the resistance of the malaria parasite, *P. falciparum* to antimalarial drugs. Reports of chloroquine resistant strains of *P. falciparum* are being documented in all regions of the world where malaria is endemic. Resistance to antimalarial drugs other than chloroquine is also occurring at an alarming rate. Therapeutic efficacy studies remain the gold standard for guiding drug policy and should be undertaken every 2 years (WHO, 2013). All malaria endemic countries are therefore recommended to assess the level of antimalarial drug resistance using WHO recommended protocols, and change their drug policy if significant resistance is documented (WHO, 2013). Resistance to chloroquine and sulfadoxine-pyrimethamine originated on the Thai- Cambodian border and subsequently spread across Asia and Africa, causing millions of deaths (Roper *et al.,* 2004). Studies conducted in western Cambodia showed that there is a reduced *in-vivo* susceptibility of ***P. falciparum*** to artesunate monotherapy, characterised by slow parasite clearance without concomitant reduction of *in-vitro* susceptibility (Dondorp *et al.,* 2009). Resistance in most cases occurs as a result of exposure of *P. falciparum* to sub-therapeutic concentrations of antimalarial drugs which might arise from suboptimum dosing (Barnes *et al.,* 2006), the use of ineffective, substandard, or counterfeit drugs (Newton ***et al.,*** 2008) or from failure to complete a full treatment course (patient poor compliance). As in